STEM CELL LABORATORY (STCL)

DOCUMENT NUMBER: STCL-PROC-015 JA6

DOCUMENT TITLE:

CliniMACS User Manual US Edition JA6

DOCUMENT NOTES:

Document Information

Revision: 01	**Vault:** STCL-Processing-rel
Status: Release	**Document Type:** STCL

Date Information

Creation Date: 26 Jun 2023	**Release Date:** 28 Jun 2023
Effective Date: 28 Jun 2023	**Expiration Date:**

Control Information

Author: Barbara Waters-Pick (WATER002)	**Owner:** Barbara Waters-Pick (WATER002)
Previous Number: None	**Change Number:** STCL-CCR-548

Revision History Form

Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**
Delivery no.		**Exchange of pages:**	**Date**		**Signature**

CliniMACS®

User Manual
US Edition

- Software 2.40 -

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany

Distributed by:
Miltenyi Biotec Inc.
2303 Lindbergh Street
Auburn, CA 95602, USA

US Customer Service:
Phone +1-800-810-3135
Prompt 1: Technical Support including Emergency Support
Prompt 2: Complaint Registration
Prompt 3: Customer Service

Issued:
August 2015

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Table of contents

1 General 1
1.1 Introduction 1
1.2 Technical Support and Service Information 2
1.3 The CliniMACS® plus Instrument 3
1.3.1 Intended purpose 3
1.3.2 Warnings and precautions 3
1.3.3 Specifications 5
1.3.4 Guidance and manufacturer's declaration on electromagnetic compatibility 7
1.3.5 Unpacking 11
1.3.6 Cleaning and maintenance 14
1.3.7 Instrument disposal 14
1.3.8 Description of the CliniMACS® plus Instrument (Model CS2-CE/UL) 15
1.3.9 Installation of the CliniMACS® plus Instrument 17
1.3.10 General menus to set-up the CliniMACS® plus Instrument 18

2 Glossary 1
2.1 Glossary of symbols 1
2.2 Glossary of terms 4

3 Customer information 1
3.1 MACS® Magnetic Cell Separation 1
3.2 The CliniMACS® Plus System 1
3.3 CliniMACS® Reagents and Biotin Conjugates 1
3.4 CliniMACS® Tubing Sets 2
3.5 CliniMACS® PBS/EDTA Buffer 4
3.6 Additional materials required 5
3.7 Equipment required 6
3.8 General warnings and precautions 7
3.9 Labeling of cells with CliniMACS® Reagents 10
3.10 High-gradient magnetic cell separation 11
3.11 CliniMACS® Separation strategies 12

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

4 Four STEPS to your target cells 1

CliniMACS® CD34 Reagent 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® CD34 Reagent 2

II. First actions required 4
CliniMACS® Materials required 4
Additional materials required 4
Equipment required 5
Preparation of the CliniMACS® PBS/EDTA Buffer 5
Labeling and preparation of bags 5

III. Leukapheresis product 6
Analysis of leukapheresis product 6
Transfer of leukapheresis product into Cell Preparation Bag 7
Dilution of leukapheresis product 8
Centrifugation 9
Volume adjustment 9

IV. Magnetic labeling of cells 11
Incubation with the CliniMACS® CD34 Reagent 11
Removal of excess reagent 12

Worksheet for preparation and enrichment of CD34 positive cells 1

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® CD133 Reagent (Leukapheresis Product) 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® CD133 Reagent 2

II. First actions required 4
CliniMACS® Materials required 4
Additional materials required 4
Equipment required 5
Preparation of the CliniMACS® PBS/EDTA Buffer 5
Labeling and preparation of bags 5

III. Leukapheresis product 6
Analysis of leukapheresis product 6
Transfer of leukapheresis product into Cell Preparation Bag 7
Dilution of leukapheresis product 8
Centrifugation 9
Volume adjustment 9

IV. Magnetic labeling of cells 11
Incubation with the CliniMACS® CD133 Reagent 11
Removal of excess reagent 12

Worksheet for preparation and enrichment of CD133 positive cells from leukapheresis product 1

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® CD14 Reagent 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® CD14 Reagent 2

II. First actions required 4
CliniMACS® Materials required 4
Additional materials required 5
Equipment required 5
Preparation of the CliniMACS® PBS/EDTA Buffer 7
Labeling and preparation of bags 7

III. Leukapheresis product 8
Analysis of leukapheresis product 8
Transfer of leukapheresis product into Cell Preparation Bag 9
Dilution of leukapheresis product 10
Centrifugation 11
Volume adjustment 11

IV. Magnetic labeling of cells 13
Incubation with the CliniMACS® CD14 Reagent 13
Removal of excess reagent 14

Worksheet for preparation and enrichment of CD14 positive cells 1

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® Anti-Biotin Reagent 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® Anti-Biotin Reagent 2
II. First actions required 4
CliniMACS® Materials required 4
Additional materials required 4
Equipment required 5
Preparation of the CliniMACS® PBS/EDTA Buffer 7
Labeling and preparation of bags 7
III. Cellular starting product 8
Analysis of cellular starting product 8
Transfer cellular starting product into Cell Preparation Bag 9
Dilution of Cellular starting product 10
Centrifugation 11
Primary Antibody Labeling 11
Removal of excess primary reagent: 1st Wash 12
Volume Adjustment 13
IV. Magnetic Labeling of the Cells 14
Incubation with CliniMACS®Anti-Biotin Reagent 14
Removal of Excess Reagent: 2nd Wash 15

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® CD56 Reagent 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® CD56 Reagent 2

II. First actions required 4
CliniMACS® Materials required 4
Additional materials required 4
Equipment required 5
Preparation of the CliniMACS® PBS/EDTA Buffer 5
Labeling and preparation of bags 5

III. Leukapheresis product 6
Analysis of leukapheresis product 6
Transfer Leukapheresis Product into Cell Preparation Bag 7
Dilution of Leukapheresis Product 8
Centrifugation 9
Volume Adjustment 9

IV. Magnetic Labeling of the Cells 11
Incubation with CliniMACS® CD56 Reagent 11
Removal of Excess Reagent 12

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® CD3 Reagent 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® CD3 Reagent 2
II. First actions required 4
CliniMACS® Materials required 4
Additional materials required 4
Equipment required 5
Preparation of the CliniMACS® PBS/EDTA Buffer 7
Labeling and preparation of bags 7
III. Leukapheresis product 8
Analysis of leukapheresis product 8
Transfer leukapheresis product into Cell Preparation Bag 9
Dilution of leukapheresis product 10
Centrifugation 11
Volume adjustment 11
IV. Magnetic labeling of the cells 13

CliniMACS® CD3/56 Reagent Combination 1

I. General information 1

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® CD19 Reagent 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® CD19 Reagent 2

II. First actions required 4
CliniMACS® Materials required 4
Additional materials required 4
Equipment required 5
Preparation of the CliniMACS® PBS/EDTA Buffer 6
Labeling and preparation of bags 6

III. Leukapheresis product 7
Analysis of leukapheresis product 7
Transfer leukapheresis product into Cell Preparation Bag 8
Dilution of leukapheresis product 9
Centrifugation 10
Volume adjustment 10

IV. Magnetic labeling of the cells 12
Incubation with the CliniMACS® CD19 Reagent 12
Removal of excess reagent 13

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® CD3/CD19 Reagent Combination 1

I. General information, warnings, and precautions 1
General information 1
Warnings and precautions 1
CliniMACS® CD3/CD19 Reagent combination 2

II. First actions required 2
CliniMACS® Materials required 2
Additional materials required 3
Equipment required 3
Preparation of the CliniMACS® PBS/EDTA Buffer 5
Labeling and preparation of bags 5

III. Leukapheresis product 6
Analysis of leukapheresis product 6
Transfer leukapheresis product into Cell Preparation Bag 7
Dilution of leukapheresis product 8
Centrifugation 9
Volume adjustment 9

IV. Magnetic labeling of the cells 11
Incubation with clinical grade immunoglobulin 11
Incubation with the CliniMACS® CD3 and the CliniMACS CD19 Reagent 11
Removal of excess reagent 12

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® CD8 Reagent 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® CD8 Reagent 2

II. First actions required 4
CliniMACS® Materials required 4
Additional materials required 5
Equipment required 5
Preparation of the CliniMACS® PBS/EDTA Buffer 7
Labeling and preparation of bags 7

III. Leukapheresis product 8
Analysis of leukapheresis product 8
Transfer leukapheresis product into Cell Preparation Bag 9
Dilution of leukapheresis product 10
Centrifugation 11
Volume adjustment 11

IV. Magnetic labeling of the cells 13
Incubation with the CliniMACS® CD8 Reagent 13
Removal of excess reagent 14

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® Cytokine Capture System (IFN-gamma) 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® Cytokine Capture System (IFN-gamma) 2

II. First actions required 5
CliniMACS® Materials required 5
Additional materials required 5
Equipment required 6
Preparation of the CliniMACS® PBS/EDTA Buffer 6
Labeling and preparation of bags 7

III. Cellular starting product 8
Transfer of cellular starting product into Cell Preparation Bag No. 1 9
Dilution of cellular starting product 10
Centrifugation 11
Volume adjustment 12

IV. Labeling of cells 13
In vitro restimulation 13
Labeling with the CliniMACS® IFN-gamma Catchmatrix Reagent 13
Secretion phase 15
Magnetic labeling of cells with the CliniMACS® IFN-gamma Enrichment Reagent 17

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® CD25 Reagent 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® CD25 Reagent 2

II. First actions required 4
CliniMACS® Materials required 4
Additional materials required 5
Equipment required 5
Preparation of the CliniMACS® PBS/EDTA Buffer 7
Labeling and preparation of bags 7

III. Leukapheresis product 8
Analysis of leukapheresis product 8
Transfer leukapheresis product into Cell Preparation Bag 9
Dilution of leukapheresis product 10
Centrifugation 11
Volume adjustment 11

IV. Magnetic labeling of the cells 13
Incubation with the CliniMACS® CD25 Reagent 13
Removal of excess reagent 14

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® CD4 Reagent 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® CD4 Reagent 2

II. First actions required 4
CliniMACS® Materials required 4
Additional materials required 5
Equipment required 5
Preparation of the CliniMACS® PBS/EDTA Buffer 7
Labeling and preparation of bags 7

III. Leukapheresis product 8
Analysis of leukapheresis product 8
Transfer leukapheresis product into Cell Preparation Bag 9
Dilution of leukapheresis product 10
Centrifugation 11
Volume adjustment 11

IV. Magnetic labeling of the cells 13
Incubation with the CliniMACS® CD4 Reagent 13
Removal of excess reagent 14

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® CD133 Reagent (Bone Marrow) 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® CD133 Reagent 2

II. First actions required 4
CliniMACS® Materials required 4
Additional materials required 4
Equipment required 5
Preparation of the CliniMACS® PBS/EDTA Buffer 5
Labeling and preparation of bags 5

III. Bone marrow aspirate 6
Analysis of bone marrow aspirate 6
Filtration of Bone Marrow Aspirate and transfer into Cell Preparation Bag 7
Dilution of bone marrow aspirate 8
Centrifugation 9
Volume adjustment 9

IV. Magnetic labeling of cells 11
Incubation with clinical grade immunoglobulin 11
Incubation with the CliniMACS® CD133 Reagent 11
Removal of excess reagent:
Wash No. 1 and Wash No. 2 12

Worksheet for preparation and enrichment of CD133 positive cells from bone marrow aspirate 1

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® TCRα/β-Biotin 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® TCRα/β-Biotin 2

II. First actions required 4
CliniMACS® Materials required 4
Additional materials required 4
Equipment required 5
Preparation of the CliniMACS® PBS/EDTA Buffer 5
Labeling and preparation of bags 5

III. Leukapheresis product 6
Analysis of leukapheresis product 6
Transfer leukapheresis product into Cell Preparation Bag 7
Dilution of leukapheresis product 8
Centrifugation 9
Volume adjustment 9

IV. Labeling of the cells with the CliniMACS® TCRα/β-Biotin 11
Incubation with CliniMACS® TCRα/β-Biotin 11
Removal of excess conjugate 12

V. Magnetic labeling of the cells with the CliniMACS® Anti-Biotin Reagent 14
Incubation with CliniMACS® Anti-Biotin Reagent 14
Removal of excess reagent 15

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® CD45RA Reagent 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® CD45RA Reagent 2

II. First actions required 4
CliniMACS® Materials required 4
Additional materials required 4
Equipment required 4
Preparation of the CliniMACS® PBS/EDTA Buffer 5
Labeling and preparation of bags 5

III. Leukapheresis product 6
Analysis of leukapheresis product 6
Transfer leukapheresis product into Cell Preparation Bag 7
Dilution of leukapheresis product 8
Centrifugation 9
Volume adjustment 9

IV. Magnetic labeling of the cells 11
Incubation with CliniMACS® CD45RA Reagent 11
Removal of excess reagent 12

Worksheet for preparation and depletion of CD45RA positive cells 1

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® CD271 Reagent (Bone marrow) 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® CD271 Reagent 2

II. First actions required 4
CliniMACS® Materials required 4
Additional materials required 5
Equipment required 5
Preparation of the CliniMACS® PBS/EDTA Buffer 6
Labeling and preparation of bags 6

III. Bone marrow aspirate 7
Analysis of bone marrow aspirate 7
Filtration of bone marrow aspirate and transfer into Cell Preparation Bag 8
Dilution of bone marrow aspirate 9
Centrifugation 10
Volume adjustment 11

IV. Magnetic labeling of the cells 13
Incubation with CliniMACS® CD271 Reagent 13
Removal of excess reagent 14

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Table of contents (continued)

STEP 1: Cell preparation and magnetic labeling (continued)

CliniMACS® CD62L Reagent 1

I. General information, warnings and precautions 1
General information 1
Warnings and precautions 2
CliniMACS® CD62L Reagent 2

II. First actions required 4
CliniMACS® Materials required 4
Additional materials required 5
Equipment required 5
Preparation of the CliniMACS PBS/EDTA Buffer 7
Labeling and preparation of bags 7

III. Leukapheresis product 8
Analysis of leukapheresis product 8
Transfer of leukapheresis product into Cell Preparation Bag 9
Dilution of leukapheresis product 10
Centrifugation 11
Volume adjustment 11

IV. Magnetic labeling of cells 13
Incubation with the CliniMACS® CD62L Reagent 13
Removal of excess reagent 14

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Table of contents (continued)

STEP 2: Start of the CliniMACS®plus Instrument and choice of separation program

CD34 SELECTION 1/2 1

Switch-on of the CliniMACS® plus Instrument 1

Choice of separation program CD34 SELECTION 1/2 1

CD133 SELECTION 1/2 1

Switch-on of the CliniMACS® plus Instrument 1

Choice of separation program CD133 SELECTION 1/2 1

ENRICHMENT 1.1 1

Switch-on of the CliniMACS® plus Instrument 1

Choice of separation program ENRICHMENT 1.1 1

Sample parameter input 3

ENRICHMENT 3.2 1

Switch-on of the CliniMACS® plus Instrument 1

Choice of separation program ENRICHMENT 3.2 1

DEPLETION 2.1 1

Switch-on of the CliniMACS® plus Instrument 1

Choice of separation program DEPLETION 2.1 1

Sample parameter input 3

DEPLETION 3.1 1

Switch-on of the CliniMACS® plus Instrument 1

Choice of separation program DEPLETION 3.1 1

Sample parameter input 3

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Table of contents (continued)

STEP 3: Installation of CliniMACS® Tubung Sets

CliniMACS® Tubing Set TS and CliniMACS Tubing Set LS 1

Preparation for tubing set installation 1
Attach Cell Collection Bag 1
Attach Priming Waste Bag and insert pre-column 2
Insert separation column and load valve no. 5 4
Load valves nos. 1, 2, 3, and 4 5
Load pump tubing 6
Load valves nos. 7 and 8 7
Load valves nos. 6, 9, 10, and 11 7
Recheck all tubing and attachments 8
Seating of valves 8
Attach CliniMACS® PBS/EDTA Buffer 9
Start priming 10
Check during the priming 10
Final check of all tubing and attachments 10
Integrity test 11
Connect Cell Preparation Bag 13
Final check of the liquid sensor 13

Alternative installation of CliniMACS® Tubing Sets 1

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Table of contents (continued)

CliniMACS® Depletion Tubing Set (REF 261-01) 1

Preparation for tubing set installation 1
Attach Non-Target Cell Bag, Reapplication Bag, and insert separation column 2
Load valves nos. 1, 2, 3, 4, and 5 4
Load pump tubing 5
Load valves nos. 6, 7, 8, 9, and 10 6
Recheck all tubing and attachments 6
Seating of valves 6
Attach CliniMACS® PBS/EDTA Buffer 7
Start priming 8
Check during the priming 8
Final check of all tubing and attachments 8
Integrity test 9
Connect Cell Preparation Bag 11
Final check of the liquid sensor 12

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Table of contents (continued)

STEP 4: CliniMACS® Separation

CD34 SELECTION 1/2 1

Separation procedure 1
Disconnect bags and record process code 3
Unload tubing set and shutdown 4
Analysis of cells 4

CD133 SELECTION 1/2 1

Separation procedure 1
Disconnect bags and record process code 3
Unload tubing set and shutdown 4
Analysis of cells 4

ENRICHMENT 1.1 1

Separation procedure 1
Disconnect bags and record process code 3
Unload tubing set and shutdown 4
Analysis of cells 4

ENRICHMENT 3.2 1

Separation procedure 1
Disconnect bags and record process code 3
Unload tubing set and shutdown 4
Analysis of cells 4

DEPLETION 2.1 1

Separation procedure 1
Disconnect bags and record process code 3
Unload tubing set and shutdown 4
Analysis of cells 4

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

DEPLETION 3.1 1

Separation procedure 1

Disconnect bags and record process code 3

Unload tubing set and shutdown 4

Analysis of cells 4

5 Troubleshooting 1

5.1 Preparation of the leukapheresis product 1

5.2 CliniMACS® plus Instrument and CliniMACS Tubing Sets 1

5.3 Automated cell separation 3

5.4 Cell separation performance 13

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

1 General

1.1 Introduction

The [1]CliniMACS System offers a set of tools making high quality standard cell separations available for therapeutic applications. The CliniMACS Plus System is based on the magnetic cell separation technology (MACS® Technology) developed by Miltenyi Biotec GmbH. Miltenyi has made this technology available for clinical applications meeting the requirements of European Regulatory Standards and the U.S Food and Drug Administration.

The CliniMACS Components - CliniMACS plus Instrument, CliniMACS Reagents, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer - enable the user to enrich or deplete human cells from heterogeneous hematologic cell populations under GMP conditions.

In the US, the CliniMACS Plus System components including the CliniMACS Reagents are available for use only under an approved Investigational New Drug (IND) application or Investigational Device Exemption (IDE).

Note

Chapter 4, "Four STEPS to your target cells", provides a detailed description regarding how to use the CliniMACS User Manual.

Limited Warranty

Should the CliniMACS Plus System be used in a manner not explicitly described in this manual, all warranties will be null and void.

1 CliniMACS and MACS are registered trademarks of Miltenyi Biotec GmbH, Germany.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

1.2 Technical Support and Service Information

CliniMACS Technical Support

For any information regarding the CliniMACS Plus Systems and their components, please contact CliniMACS Customer Service:

Customer Service:
Phone +1-800-810-3135

Miltenyi Biotec Inc.
2303 Lindbergh Street
Auburn CA 95602, USA

Phone +1-800-367-6227
Prompt 1: Technical Support, including Emergency Support
Prompt 2: Complaint Registration
Prompt 3: Customer Service

macs@miltenyibiotec.de
www.miltenyibiotec.com

CliniMACS plus Instrument information

Please record the model and serial number located on the back of the CliniMACS plus Instrument below. The operator should refer to these numbers when calling to obtain information or request service on the instrument.

Model no.: _____

Serial no.: _____

Software version: _____

The software version is shown during the start-up phase of the CliniMACS plus Instrument (see screen no. 1.1, chapter 1.8).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

1.3 The CliniMACS® plus Instrument

1.3.1 Intended purpose

The CliniMACS® plus Instrument is an electromechanical device intended to separate human cells from heterogeneous hematologic cell populations in combination with the CliniMACS Reagents, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer only.

1.3.2 Warnings and precautions

1. Read and observe all operating instructions carefully (see Fig. 1.1).

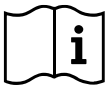


Fig. 1.1: Consult instructions for use.

2. Equipment safety will be compromised if it is not used according to the manufacturer's instructions.

3. Risk of serious personal injury! The CliniMACS plus Instrument is equipped with an extremely powerful magnet. Keep any magnetic information carriers (such as credit cards and magnetic tapes), any electronic equipment (such as hearing aids, pacemakers, cerebral/brain shunts, measuring and control instruments, computers, and watches) and magnetizable tools and objects at a distance of at least 30 cm from the device. These items may be affected or damaged by the magnetic field (see Fig. 1.2).

Fig. 1.2: Strong magnetic field

4. Medical electrical equipment needs special precautions regarding electromagnetic compatibility (EMC) and needs to be installed and put into service according to the EMC information provided in the accompanying documents. Portable and mobile RF communications equipment can affect medical electrical equipment.

5. The CliniMACS plus Instrument is a protection class I device and may only be plugged into an outlet with a grounded connection.

6. Before cleaning or maintenance of the CliniMACS plus Instrument, the power cord cable should be disconnected.

7. To disconnect the CliniMACS plus Instrument from the power supply unplug the power cord. Only use the originally supplied power cord. Ensure that the main switch as well as the connector for the power cable are easily accessible and located as close to the operator of the instrument as possible.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

8. To prevent the risk of an electric shock, do not remove the back cover of the CliniMACS plus Instrument. The instrument may be opened and any spare parts may be exchanged by authorized personnel only.

9. Movement or vibration may affect the CliniMACS plus Instrument. Do not place the instrument next to any equipment that vibrates or can cause the instrument to move.

10. There are no components which can be serviced or calibrated by the operator.

11. Never leave the CliniMACS plus Instrument unattended during a run. If an error occurs, the cell separation can be interrupted at the current step and the operator will have 600 seconds to correct certain errors. If the instrument has not been restarted after this time period, the run will be aborted.

12. The pump door should not be left open at any time during a run. If left open for more than 600 seconds, the run in process will be aborted.

13. Do not open the door of the peristaltic pump when it is moving. Keep away from all moving parts.

14. Fluid containers must be handled with caution when near the CliniMACS plus Instrument. Avoid spills. Do not operate the instrument if it has been exposed to moisture. Avoid ingress of any liquid into the valves.

15. After running a patient sample and prior to decontamination, the CliniMACS plus Instrument should be treated as a biohazard.

16. The CliniMACS plus Instrument may be used repeatedly. It is not intended for disposal after single use. It must be returned to Miltenyi Biotec for final disposal.

17. Only supplies (e.g. CliniMACS Tubing Sets) recommended by the manufacturer may be used.

18. Allow sufficient air circulation around the instrument – at least 15 cm on all sides – during operation to ensure adequate cooling. In the absence of adequate circulation, ambient air may not cool the instrument to acceptable operating temperatures.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

1.3.3 Specifications

The CliniMACS plus Instrument has been investigated by Underwriter Laboratories in accordance with the standards

UL 61010-1,
CAN/CSA-C22.2 No. 61010-1, and
IEC 61010-1,

and complies with all essential requirements of the European directive:

93/42/EEC (Medical Device Directive).

The instrument is in conformity with the harmonized standards EN 61010-1, and EN 60601-1-2.

Fig. 1.3: UL listing mark, listed as laboratory equipment

Fig. 1.4: European conformity approval

Technical specifications	
Model	CliniMACS plus Instrument, Model CS2-CE/UL (REF 151-01)
Dimensions	Width: 70 cm Height: 90-140 cm Depth: 60 cm
Weight	35 kg
Input voltage	100-240 VAC (Single phase alternating current)
Power consumption	180 VA
Power source	An uninterruptible power source is recommended (reliable, noise free utility). Recommended UPS: APC Smart-UPS 1500VA USB & Serial 230 V, manufactured by APC (American Power Conversion) or equivalent.
Instrument power	Instrument power inlet IEC-320-C13 A country specific power cord is supplied with the CliniMACS plus Instrument.
Frequency	50/60 Hz
Fuses	2× T4A/250V, 5 × 20 mm Use only fuses with UL and European approvals, acc. to IEC 127-2/III, EN 60127-2/III, DIN 41662.
Operation conditions	+10 °C to +30 °C with 0% to 85% humidity at an altitude of max. 2000 m. Supply voltage fluctuations up to ±10% of the nominal voltage. Transient over-voltages present on the mains supply: category II. The instrument is suitable for rated pollution degree 2. The instrument is intended for indoor use only.
Storage conditions	–10 °C to +60 °C with 0% to 85% humidity, when contained and sealed in the outer packaging provided by the manufacturer

Table 1.1: Technical specification of the CliniMACS plus Instrument

Caution

Changes or modifications not expressly approved by the manufacturer of the CliniMACS Plus System could invalidate the user's authority to operate this system.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

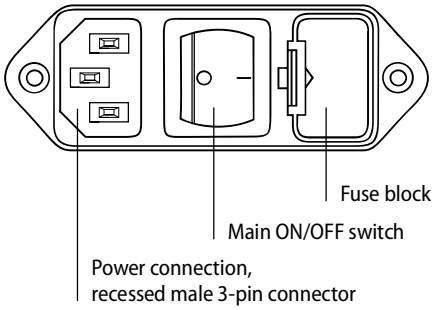


Fig. 1.5: Rear view of the CliniMACS plus Instrument.

- **Protection class:**
The CliniMACS plus Instrument is a protection class I device (acc. to DIN 61140) and may only be plugged into an outlet with a grounded conductor. The protection category according to DIN EN 60529 is IPX 0.

- **Power connection (see fig 1.5):**
The power connection module is located at the rear of the CliniMACS plus Instrument. Viewed from behind, the connection consists of three sections. The left section is the recessed male 3-pin connector to which the power cord is attached. The center section is the main ON/OFF switch. When positioned to the left, the switch is 'OFF' (O). When positioned to the right, the switch is 'ON' (I).

The right section is the fuse box. The CliniMACS plus Instrument must be unplugged and switched off before opening the fuse box. To open it, a thin-bladed screwdriver is inserted into the slot and twisted to release the catch. To replace the fuses, remove the fuses from the rear, insert new fuses and slide the module back in until the latch clicks to the closed position. The module will only slide in one direction. Only fuses with UL and European approvals are to be used.

LPT1

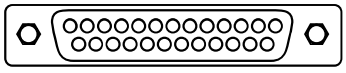


Fig. 1.6: Parallel printer port, SUB D 25, female connector.

- **Printer port:**
There is a printer port (see fig. 1.6) situated at the rear of the CliniMACS plus Instrument next to the power connection. However, a printer is currently not supported. Please do not connect any printer.

- **Interferences:**
This equipment has been tested and found to comply with the limits for a class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

  - Redirect or relocate the receiving antenna.
  - Increase the space between the equipment and receiver.
  - Connect the equipment to an outlet which is not on the same circuit as the receiver.
  - Consult the dealer or an experienced radio/TV technician for help.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

1.3.4 Guidance and manufacturer's declaration on electromagnetic compatibility

Medical electrical equipment needs special precautions regarding electromagnetic compatibility (EMC) and needs to be installed and put into service according to the EMC information provided in the accompanying documents. Portable and mobile RF communications equipment can affect medical electrical equipment.

EMC compliance with IEC 60601-1-2:2007 has been attested for the provided power cable. The use of other power cables may result in increased electromagnetic emissions or decreased immunity of the CliniMACS plus Instrument. If the provided power cable is missing, please contact Miltenyi Biotec for information on a replacement part.

Guidance and manufacturer's declaration – Electromagnetic emissions		
The CliniMACS plus Instrument is intended for use in the electromagnetic environment specified below. The customer or the user of the CliniMACS plus Instrument should assure that it is used in such an environment.		
Emissions test	**Compliance**	**Electromagnetic environment guidance**
RF Emissions CISPR 11	Group 1	The CliniMACS plus Instrument uses RF energy only for its internal function. Therefore, its RF emissions are very low and are not likely to cause any interference in nearby electronic equipment.
RF Emissions CISPR 11	Class B	The CliniMACS plus Instrument is suitable for use in all establishments, including domestic establishments and those directly connected to the public low voltage power supply network that supplies buildings used for domestic purposes.
Harmonic emissions IEC 61000-3-2	Class A	
Voltage fluctuations/ Flicker emissions IEC 61000-3-3	Complies	

Table 1.2: Guidance and manufacturer's declaration – Electromagnetic emissions

The CliniMACS plus Instrument should not be used adjacent to or stacked with other equipment and that if adjacent or stacked use is necessary, the CliniMACS plus Instrument should be observed to verify normal operation in the configuration in which it will be used.

Based on technical limitations of the internal power supply voltage, interruptions on power supply input lines for longer than 10 ms may lead to cessation of the separation process (power failure). The separation process cannot be resumed after a power failure. It is recommended that the CliniMACS plus Instrument is powered from an uninterruptible power supply or a battery that starts up within 10 ms.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Guidance and manufacturer's declaration – Electromagnetic immunity			
The CliniMACS plus Instrument is intended for use in the electromagnetic environment specified below. The customer or the user of the CliniMACS plus Instrument should assure that it is used in such an environment.			
Immunity test	**IEC 60601 Test level**	**Compliance level**	**Electromagnetic environment guidance**
Electrostatic discharge (ESD) IEC 61000-4-2	± 6 kV contact ± 8 kV air	± 6 kV contact ± 8 kV air	Floors should be wood, concrete or ceramic tile. If floors are covered with synthetic material, the relative humidity should be at least 30 %.
Electrical fast transient/burst IEC 61000-4-4	± 2 kV for power supply lines ± 1 kV for input/output lines	± 2 kV for power supply lines ± 1 kV for input/output lines	Mains power quality should be that of a typical commercial or hospital environment.
Surge IEC 61000-4-5	± 1 kV line(s) to line(s) ± 2 kV line(s) to earth	± 1 kV line(s) to line(s) ± 2 kV line(s) to earth	Mains power quality should be that of a typical commercial or hospital environment.
Voltage dips, short interruptions and voltage variations on power supply input lines IEC 61000-4-11	<5 % U_T (>95 % dip in U_T) for 0.5 cycle 40 % U_T (60 % dip in U_T) for 5 cycles 70 % U_T (30 % dip in U_T) for 25 cycles <5 % U_T (>95 % dip in U_T) for 5 s	<5 % U_T (>95 % dip in U_T) for 0.5 cycle 40 % U_T (60 % dip in U_T) for 5 cycles 70 % U_T (30 % dip in U_T) for 25 cycles	Mains power quality should be that of a typical commercial or hospital environment but if the user of the CliniMACS plus Instrument requires continued operation during power mains interruptions longer than 10 ms, it is recommended that the CliniMACS plus Instrument be powered from an uninterruptible power supply or a battery that starts up within 10 ms.
Power frequency (50/60 Hz) magnetic field IEC 61000-4-8	3 A/m	3 A/m	Power frequency magnetic fields should be at levels characteristic of a typical location in a typical commercial or hospital environment.
NOTE U_T is the a.c. mains voltage prior to application of the test level.			

Table 1.3: Guidance and manufacturer's declaration – Electromagnetic immunity

The performance of the device that was determined to be essential performance:

1. Establishing and maintaining the intended flow path.
2. Delivering the specified flow.
3. Setting the necessary magnetic field in the separation column.
4. Displaying user information on progress of the process.
5. Running the sequence as intended.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Guidance and manufacturer's declaration – Electromagnetic immunity			
The CliniMACS plus Instrument is intended for use in the electromagnetic environment specified below. The customer or the user of the CliniMACS plus Instrument should assure that it is used in such an environment.			
Immunity test	**IEC 60601 Test level**	**Compliance level**	**Electromagnetic environment guidance**
Conducted RF IEC 61000-4-6	3 Vrms 150 kHz to 80 MHz	3 V	Portable and mobile RF communications equipment should be used no closer to any part of the CliniMACS plus Instrument, including cables, than the recommended separation distance calculated from the equation applicable to the frequency of the transmitter. Recommended Separation Distance $d = 1.2\sqrt{P}$ $d = 1.2\sqrt{P}$ 80 MHz to 800 MHz $d = 2.3\sqrt{P}$ 800 MHz to 2.5 GHz where P is the maximum output power rating of the transmitter in watts (W) according to the transmitter manufacturer and d is the recommended separation distance in metres (m). Field strengths from fixed RF transmitters, as determined by an electromagnetic site survey [a], should be less than the compliance level in each frequency range [b]. Interference may occur in the vicinity of equipment marked with the following symbol:
Radiated RF IEC 61000-4-3	3 V/m 80 MHz to 2.5 GHz	3 V/m	
NOTE 1: At 80 MHz and 800 MHz, the higher frequency range applies. NOTE 2: These guidelines may not apply in all situations. Electromagnetic propagation is affected by absorption and reflection from structures, objects and people.			
a Field strengths from fixed transmitters, such as base stations for radio (cellular/cordless) telephones and land mobile radios, amateur radio, AM and FM radio broadcast and TV broadcast cannot be predicted theoretically with accuracy. To assess the electromagnetic environment due to fixed RF transmitters, an electromagnetic site survey should be considered. If the measured field strength in the location in which the CliniMACS plus Instrument is used exceeds the applicable RF compliance level above, the CliniMACS plus Instrument should be observed to verify normal operation. If abnormal performance is observed, additional measures may be necessary, such as re-orienting or relocating the CliniMACS plus Instrument. b Over the frequency range 150 kHz to 80 MHz, field strengths should be less than 3 V/m.			

Table 1.4: Guidance and manufacturer's declaration – Electromagnetic immunity

Recommended separation distances between portable and mobile RF communications Equipment and the CliniMACS plus Instrument			
The CliniMACS plus Instrument is intended for use in an electromagnetic environment in which radiated RF disturbances are controlled. The customer or the user of the CliniMACS plus Instrument can help prevent electromagnetic interference by maintaining a minimum distance between portable and mobile RF communications equipment (transmitters), including RFID readers, and the CliniMACS plus Instrument – as recommended below, according to the maximum output power of the communications equipment.			
Rated maximum output power of transmitter (W)	**Separation distance according to frequency of transmitter (m)**		
	125 kHz to 80 MHz $d = 1.2 \sqrt{P}$	80 MHz to 800 MHz $d = 1.2 \sqrt{P}$	800 MHz to 2,5 GHz $d = 2.3 \sqrt{P}$
0.01	0.12	0.12	0.23
0.1	0.38	0.38	0.73
1	1.2	1.2	2.3
10	3.8	3.8	7.3
100	12	12	23
For transmitters rated at a maximum output power not listed above, the recommended separation distance d in metres (m) can be estimated using the equation applicable to the frequency of the transmitter, where P is the maximum output power rating of the transmitter in watts (W) according to the transmitter manufacturer.			
NOTE 1: At 80 MHz and 800 MHz, the separation distance for the higher frequency range applies. NOTE 2: These guidelines may not apply in all situations. Electromagnetic propagation is affected by absorption and reflection from structures, objects and people.			

Table 1.5: Recommended separation distances between portable and mobile RF communications equipment and the CliniMACS plus Instrument

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

1.3.5 Unpacking

Unpacking the CliniMACS plus Instrument should be performed by two people, according to the following instructions.

Note

Visually inspect and note any significant damage to the package before unpacking. Damage may require inspection by a representative of the shipping company.

1. Cut the plastic straps using a pair of scissors (see fig. 1.7).

Caution

Wear safety glasses. The straps are wrapped under tension.

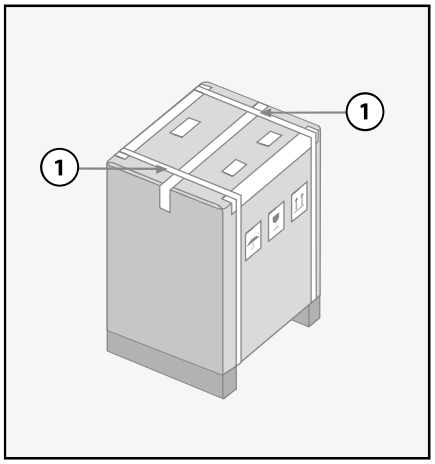


Fig. 1.7: Cut plastic straps

2. Open the top carton by cutting the adhesive tape (see fig. 1.8).

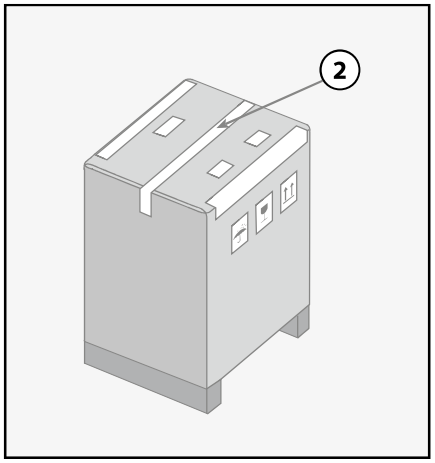


Fig. 1.8: Open top carton

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

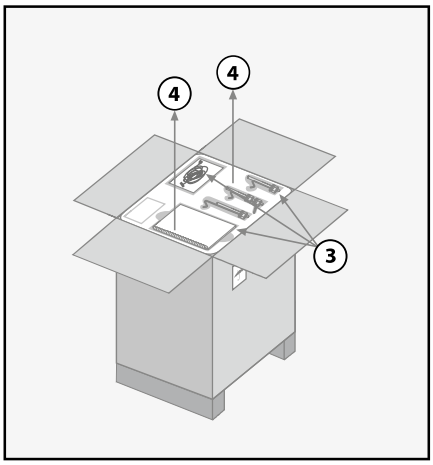


Fig. 1.9: Remove the parts and the protective foam

3. Open the carton and remove the parts (power cord, bag hangers) from the protective foam (see fig. 1.9).

4. Remove the protective foam.

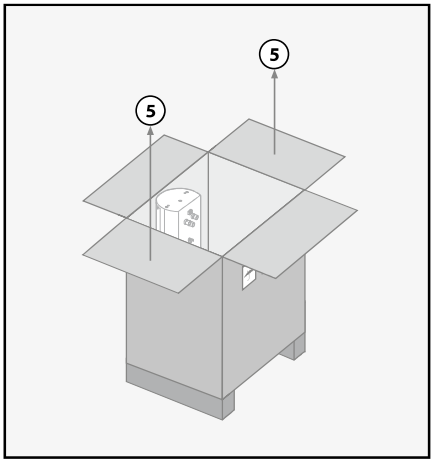


Fig. 1.10: Lift the top carton

5. Lift the top carton vertically off the pallet (see fig. 1.10).

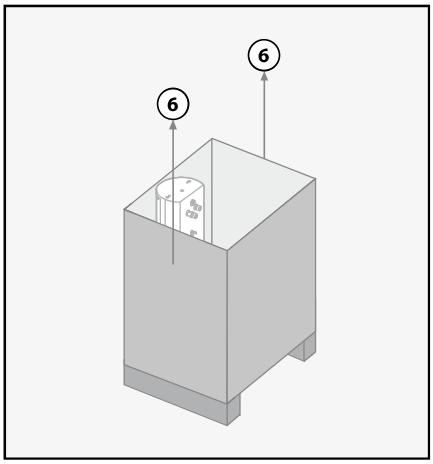


Fig. 1.11: Remove the inner carton

6. Remove the inner carton (see fig. 1.11).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

7. Unwrap the large shipping bag (see fig. 1.12).

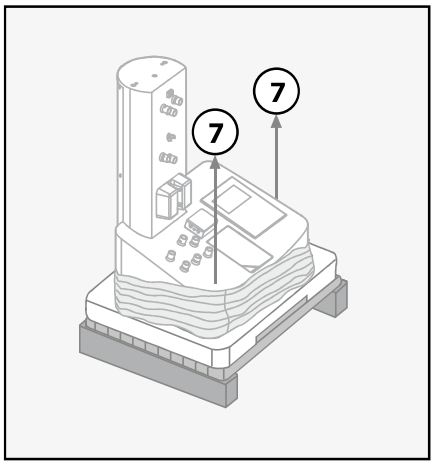


Fig. 1.12: Lift the CliniMACS plus Instrument

Caution

Two people should carefully lift the CliniMACS plus Instrument onto a flat, stable surface which is capable of supporting 100 kg. The instrument should be lifted under each of the four corners at the base of the instrument. The instrument is heavier at the back and should be stabilized while lifting it. Take care to avoid personal strain or injury.

8. To maintain ventilation, place the instrument at least 10 cm away from the wall.

Note

Do not locate the CliniMACS plus Instrument next to any vibrating equipment which might cause movement during operation.

9. Attach bag hangers. Tighten rods with clockwise twists until hand tight. The height of the bag hangers can be adjusted by pressing the bag hanger clamps (see fig. 1.13).

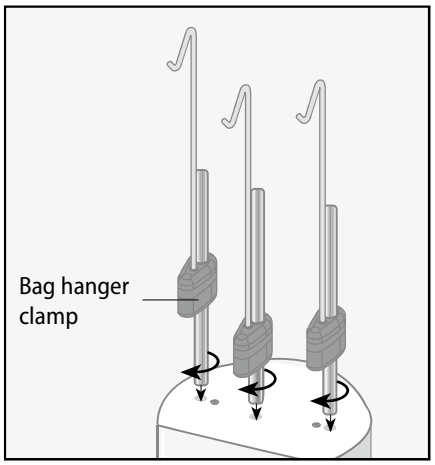


Fig. 1.13: Attach bag hangers

10. A stabilization foot (see fig. 1.14) is included with your delivery. Please keep this foot for later installation by the Miltenyi Biotec Technical Service or an authorized representative. The foot will be installed at the back of the instrument.

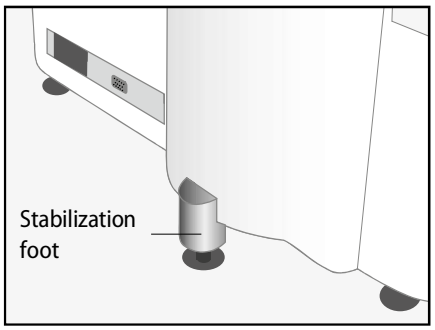


Fig. 1.14: Rear of CliniMACS plus Instrument with installed stabilization foot

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Caution

- Clean the CliniMACS plus Instrument only when it is switched off and the power cord is unplugged.
- Avoid ingress of any liquid into the valves.

1.3.6 Cleaning and maintenance

Cleaning

The surface of the CliniMACS plus Instrument should be cleaned at regular intervals and after each application with an antiseptic solution (according to standard procedures for device decontamination).

Do not use other cleaning agents or an excessive amount of water. After cleaning, dry all excess liquid from the valves, pumphead etc.

Maintenance

The CliniMACS plus Instrument does not contain operator serviceable parts. Routine and preventative maintenance procedures should be conducted by the manufacturer's authorized service personnel at least once a year. Calibration is not required.

1.3.7 Instrument disposal

Fig. 1.15: Separate collection for waste of electrical and electronic equipment

The CliniMACS plus Instrument must be separately collected according to the European directive of waste of electrical and electronic equipment (WEEE). For final disposal, the instrument must be returned to Miltenyi Biotec. Clean the instrument according to the instructions given in section "Cleaning and maintenance".

Please contact Miltenyi Biotec Technical Service for assistance prior to disposal of the instrument.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

1.3.8 Description of the CliniMACS® plus Instrument (Model CS2-CE/UL)

The CliniMACS® plus Instrument is an electromechanical device intended to separate human cells from heterogeneous hematologic cell populations in combination with the CliniMACS Reagents, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer.

The key components of the CliniMACS plus Instrument are an integrated computer, a magnetic separation unit, a peristaltic pump, a liquid sensor, and pinch valves.

The integrated microcomputer controls all electromechanical components of the instrument and directs the system to perform procedures in a standard sequence. The keypad and display guide the operator through the set-up procedure and allow monitoring of automatic system operations (see Fig 1.16).

The magnetic separation unit includes the movable permanent magnet and the selection column holder for the selection column.

During the separation, the peristaltic pump controls the flow rate through the CliniMACS Tubing Sets. The liquid sensor monitors the flow of labeled cell suspension into the tubing set. Disruption of continuous fluid flow through the sensor automatically advances the separation program to the next phase of the separation process.

Eleven pinch valves ensure controlled flow of buffer and cell suspension throughout the procedure.

The CliniMACS plus Instrument and CliniMACS Tubing Sets allow the operator to perform cell separations in a closed and sterile system.

The CliniMACS plus Instrument software offers the operator the choice between various separation programs. For further details see chapter 4.

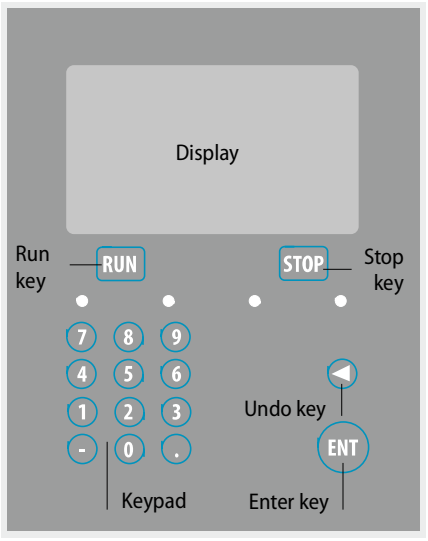


Fig. 1.16: Keypad of the CliniMACS plus Instrument

Important

- This CliniMACS User Manual only contains the instructions for **clinical applications** using the **CliniMACS Plus Reagent Systems.** In the US, the CliniMACS components are available for use only under an approved Investigational New Drug (IND) application or Investigational Device Exemption (IDE).
- For **non-clinical applications** using the **CliniMACS MicroBeads for Research Use** refer to the CliniMACS Data sheets for research use and the respective special protocols, provided by the Miltenyi Biotec Technical Support.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

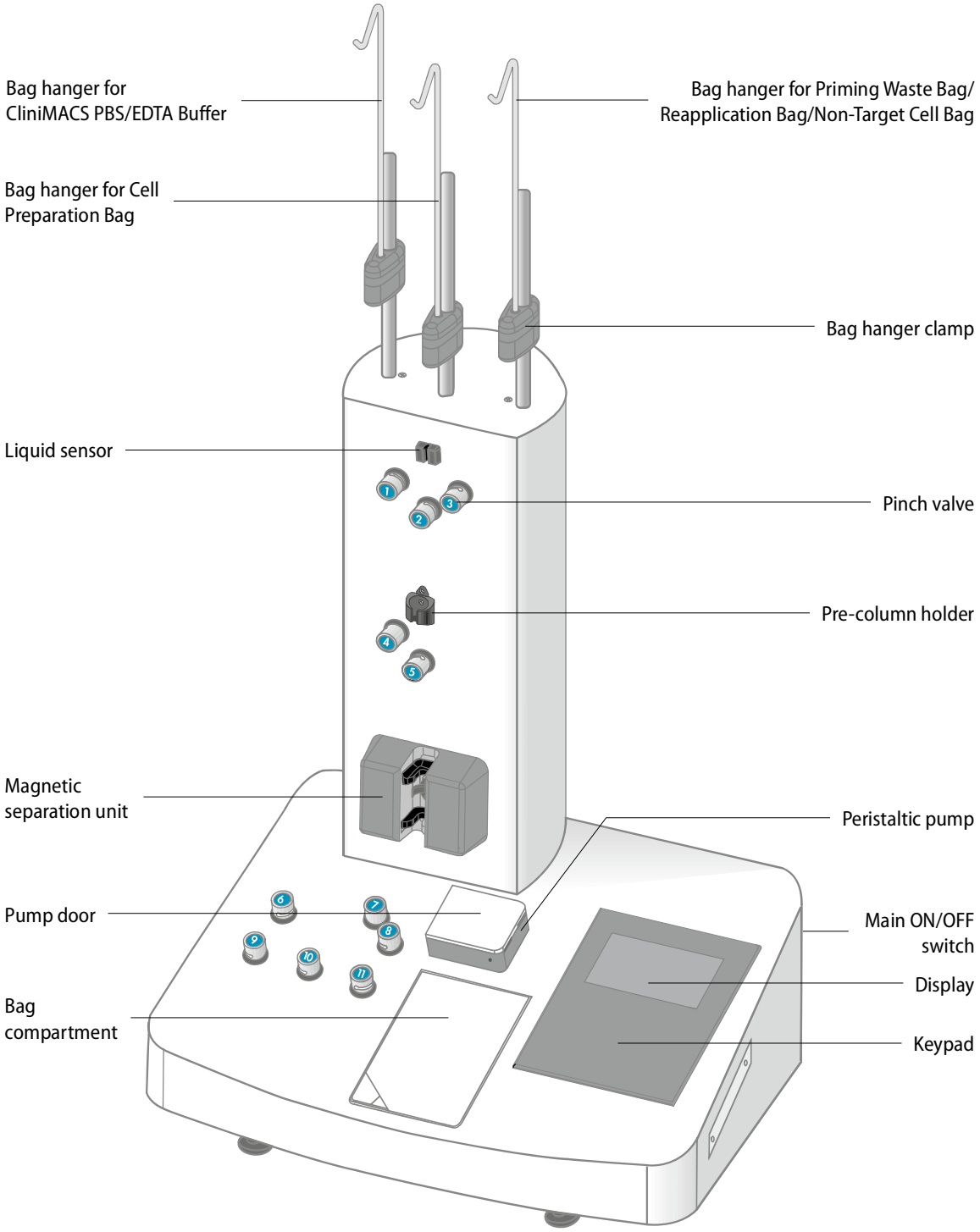


Fig. 1.17: The CliniMACS plus Instrument, Model CS2-CE/UL.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

1.3.9 Installation of the CliniMACS® plus Instrument

Connect and switch on the CliniMACS® plus Instrument

Connect the CliniMACS plus Instrument to an uninterruptable power supply using the supplied power cord. Switch on the instrument by using the ON/OFF switch (Fig. 1.17) located on the right hand back panel of the instrument.

For safety, the CliniMACS plus Instrument should be turned off after each run and the power cord cable should be disconnected during the instrument clean-up procedures.

The CliniMACS plus Instrument will perform self-checking procedures and the window will display screen no. 1.1. The program will automatically be loaded and screen no. 1.2 will appear in the display window.

If the CliniMACS plus Instrument does not start up, switch the instrument off and disconnect it from the power supply. Check the power cord connection and the fuses, which are located on the right hand back panel (see chapter 1.3.3). Then switch on the instrument again.

If the CliniMACS plus Instrument does not start correctly or the window displays an error message, note the error message number, switch the instrument off and contact Miltenyi Biotec's Technical Support.

Proper training is required to operate the CliniMACS plus Instrument. Read the instructions carefully. Further training by a Miltenyi Biotec authorized representative may be required. This will include details regarding instrument operation and sample handling.

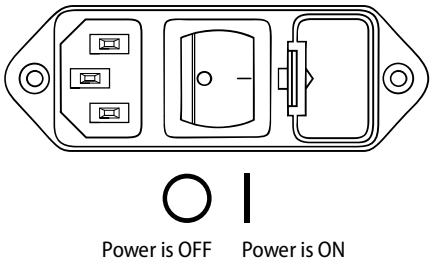
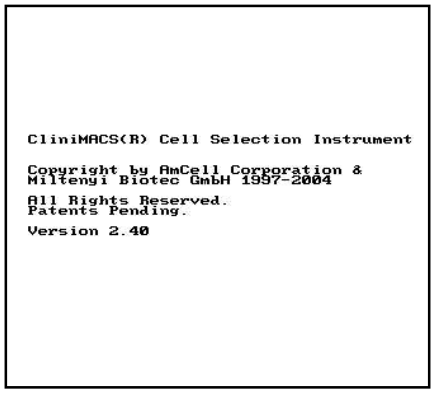


Fig. 1.18: Switch on the CliniMACS plus Instrument



Screen no. 1.1

Important

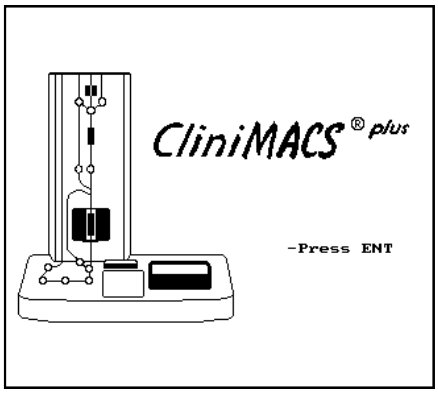
The actual CliniMACS software version number is indicated in screen no. 1.1.

Language selection and service menu

The CliniMACS plus Instument provides a menu to set-up the language and a service menu (see chapter 1.3.10 "General menus to set-up the CliniMACS plus Instrument").

To enter these menus wait until screen no. 1.2 appears in the display window and DO NOT press 'ENT' as shown on the window display (Screen no. 1.2).

To start the general menus, please refer to chapter 1.3.10 on the next page.



Screen no. 1.2

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

1.3.10 General menus to set-up the CliniMACS® plus Instrument

Language selection

Screen no. 1.3

The language selection menu allows the operator to change the language used in the display. It is possible to choose between English, German, French, Spanish, Italian and Dutch. To change the language, wait until the window displays screen no. 1.2 and DO NOT press 'ENT' then.

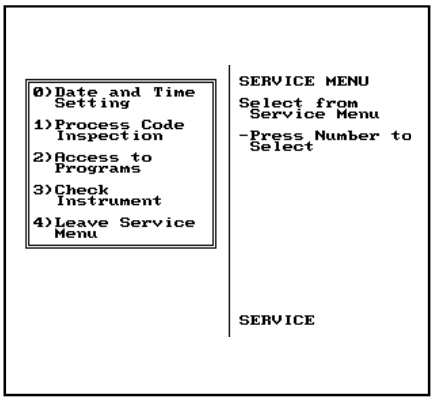
To start language selection, press [2]

The window will display screen no. 1.3 "Language selection". To select a language, press the corresponding number.

To save the language, press [ENT]

The window will automatically display screen no. 1.1 again, now in the chosen language.

Service Menu



Screen no. 1.4

The service menu contains some programs that might be useful for the operator.

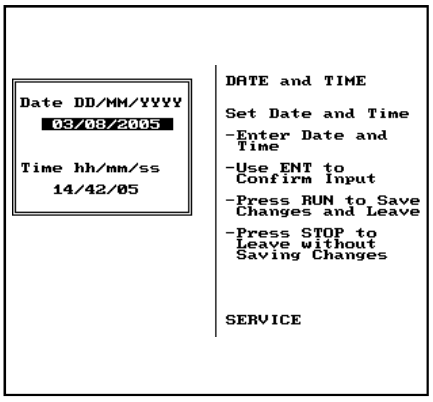
To open the service menu folder, wait until the window displays screen no. 1.2 and DO NOT press 'ENT' then.

Then press [5]

The window will display screen no. 1.4 "Service Menu".

DATE AND TIME SETTING

To set date and time, press [0]



Screen no. 1.5

Follow the instructions shown on screen no. 1.5. Date or time can be changed when the respective field is highlighted by the black bar.

To move the black bar between date and time input, press 'ENT'.

Enter the current date (order: day/month/year) and time (order: hours/minutes/seconds). A wrong input can be amended by pressing "Undo" (see fig. 1.16).

To save the data and leave, press [RUN]

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

To exit the program without saving the changes that have been done, press 'STOP'. After pressing 'RUN', the program will automatically return to the service menu.

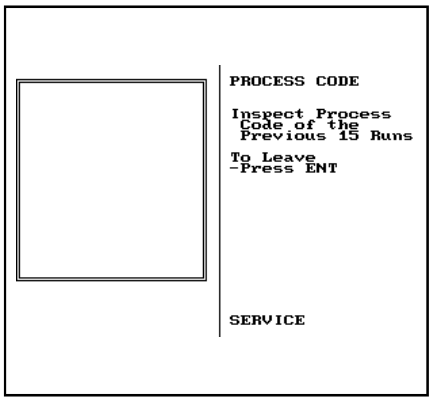
PROCESS CODE INSPECTION

The operator is able to call up the process codes of the last 15 CliniMACS Plus operations. A process code is saved when the operator has started the installation of the CliniMACS Tubing Set or when the EMERGENCY PROGRAM (see trouble shooting) has been used. Saving a process code is independent from whether the separation sequence was completed or interrupted.

To call up a process code, press 1

The process codes of the last 15 CliniMACS Plus operations are listed chronologically as shown on screen no. 1.6. The list starts with the most recent procedure.

To return to the service menu, press ENT



Screen no. 1.6

ACCESS TO PROGRAMS

This program allows for the activation of additional separation programs by Miltenyi Biotec authorized personnel.

If the operator enters this program by mistake, the operator may leave the program by pressing "STOP".

CHECK INSTRUMENT

In case of a suspected malfunction of the CliniMACS^{plus} Instrument contact Miltenyi Biotec Emergency Technical Support. If an instrument check is indicated the technical specialist will assist in performing the instrument check sequence.

If an operator has entered this program by mistake, the operator may leave the program by pressing "STOP".

To leave the service menu, press 4

The program will return to the initial screens no. 1.1 and 1.2.

2 Glossary

2.1 Glossary of symbols

CE 0123

European conformity approval with ID number 0123 (ID number of Notified Body: "TÜV SÜD Product Service GmbH, Munich"). Medical device according to MDD 93/42/EEC.

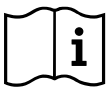
c UL us LISTED LABORATORY EQUIPMENT 40XK

UL listing mark, listed as laboratory equipment

Safety symbol: General warning sign

Safety symbol: Strong magnetic field

Caution



Consult instructions for use.

Manufacturer

Date of manufacture

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Do not re-use.

Do not use if package is damaged.

Packaging PVC free

Use-by date

Temperature limit

Contains phthalates: di(2-ethylhexyl)phthalate (DEHP)

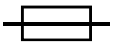
Non-pyrogenic

Non-pyrogenic fluid path

Instrument power is ON.

Instrument power is OFF.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

	Fuse
	Separate collection for waste of electrical and electronic equipment
LOT	Batch code
P/N	Part number
REF	Catalogue number (REF)
SN	Serial number
STERILE	Sterile
STERILE A	Sterilized using aseptic processing techniques
STERILE EO	Sterilized using ethylene oxide
STERILE	Sterilized using steam or dry heat

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

2.2 Glossary of terms

Anti-biotin	Anti-biotin antibody: This antibody recognizes cells which have previously been labeled with an appropriate biotinylated antibody or ligand.
Apheresis	The method of collecting blood in which whole blood is withdrawn, a desired component selected and retained, and the remainder of the blood returned to the donor.
Bag compartment	Compartment of the CliniMACS® plus Instrument in which the Negative Fraction Bag and Buffer Waste Bag are placed.
Bag hanger	Support on the CliniMACS plus Instrument to mount the Cell Preparation Bag, Non-Target Cell Bag, Priming Waste Bag, Reapplication Bag and buffer bag.
Bone marrow aspirate	Sample of bone marrow removed by the insertion of a needle into the bone and pulled into a syringe.
Buffer Waste Bag	Waste bag to collect buffer during cell separation using the CliniMACS plus Instrument.
CD3 antigen	The CD3 antigen is present on mature human T cells, thymocytes and a subset of NK cells. It is associated with the T cell receptor (TCR) and is responsible for the signal transduction of the TCR. The CD3 antigen is a complex of five invariable chains: γ, δ, ε, ζ and η. The CD3 antibody recognizes all T cells, i.e. it reacts with 70-80% of human peripheral blood lymphocytes and with 65-85% of thymocytes. The epitope recognized by the antibody is located on the ε-chain of the CD3 complex.
CD4 antigen	The CD4 antigen is expressed on most thymocytes and approximately two-thirds of peripheral blood T cells; it is also expressed on monocytes and macrophages. CD4 is an accessory molecule in the recognition of foreign antigens in association with MHC class II. Additionally, CD4 is a receptor for HIV-1.
CD8 antigen	The CD8 antigen is a disulfide-linked dimer, which exists either as a CD8 α homodimer or as a CD8 α/β heterodimer. CD8 is strongly expressed on CD8 positive T cells and thymocytes and on a subset of NK cells and CD8 positive γ/δ T cells. CD8 acts as a co-receptor with MHC class I-restricted T cell receptors in antigen recognition.
CD14 antigen	The CD14 antigen is a 53-55 kD cell membrane glycoprotein expressed on monocytes. The CD14 antigen is known as a receptor for complex of LPS and LPS binding protein (LBP).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CD19 antigen	The CD19 antigen is a critical signal transduction molecule that regulates B lymphocyte development, activation, and differentiation. As a B cell lineage marker, CD19 is expressed from the early pro-B cell stage to the B cell lymphoblast stage but the expression is downregulated upon B cell maturation to plasma cells. The CD19 antigen is further expressed on most malignant B cells and a subset of follicular dendritic cells.
CD25 antigen	The CD25 antigen, the low affinity interleukin-2 receptor alpha chain (IL-2Rα), is expressed on activated T and B cells, NK cells and monocytes, as well as on regulatory CD4 positive T cells. The CD25 antigen shows three epitope regions A, B and C. The antibody of the CliniMACS CD25 Reagent recognizes epitope A of the CD25 molecule.
CD34 antigen	The CD34 antigen is a highly glycosylated 115 kD type 1 integral membrane protein of unknown function which is expressed on 1% to 4% of normal bone marrow cells, less than 0.2% of normal peripheral blood leukocytes, on subsets of bone marrow stromal cells, and on small vessel endothelium of various tissues.
CD45RA antigen	The CD45RA antigen is expressed on naive CD4 and CD8 positive T cells, as well as on subsets of B cells, NK cells, and monocytes. It is an isoform of the common leukocyte antigen CD45, a transmembrane protein tyrosine phosphatase.
CD56 antigen	The CD56 antigen is a member of the NCAM-family (Neural Cell Adhesion Molecule-family) which is expressed within the hematopoietic system on NK, NKT cells.
CD62L antigen	The human CD62L antigen is a 74 kDa glycoprotein and a member of the selectin family of cell surface molecules. The CD62L antigen, also known as L-selectin, LECAM-1, or LAM-1, binds a series of glycoproteins including CD34, GlyCAM-1, and MAdCAM-1. CD62L is important for homing of naive lymphocytes via endothelial venules to peripheral lymph nodes and Peyer's patches. The CD62L antigen also contributes to the recruitment of leukocytes from the blood to areas of inflammation. Most hematopoietic cells express CD62L, including most peripheral blood B cells, T cells, monocytes, granulocytes, and some myeloid cells from bone marrow, and thymocytes. CD62L is continuously endoproteolytically cleaved from the cell surface of neutrophils and lymphocytes. Proteolysis is accelerated, for example, after antigenic activation of T cells.
CD133 antigen	The CD133 antigen is a 5-transmembrane cell surface antigen with a molecular weight of 117 kD and is expressed on 30-60% of all CD34 positive cells including CD34 bright cells.
CD271 antigen	The CD271 antigen, also known as low-affinity nerve growth factor receptor (LNGFR), is expressed on mesenchymal stromal cells (MSC) and can be used for the direct enrichment of these cells from bone marrow aspirate.
Cell Collection Bag	Bag into which the purified target cells are accumulated after separation.

Cell Preparation Bag	Bag into which the cellular starting product is transferred and in which magnetic labeling and washing of cells are performed.
Cellular starting product	Cell-containing product used as starting material for the CliniMACS Separation process, e.g. leukapheresis harvest, PBMCs, or bone marrow.
CliniMACS®	CliniMACS is a registered trademark of Miltenyi Biotec GmbH.
CliniMACS Anti-Biotin Reagent	Reagent for magnetic labeling of cells primarily labeled with biotinylated antibodies or ligands.
CliniMACS CD3 Reagent	Reagent for magnetic labeling of cells expressing the CD3 antigen.
CliniMACS CD4 Reagent	Reagent for magnetic labeling of cells expressing the CD4 antigen.
CliniMACS CD8 Reagent	Reagent for magnetic labeling of cells expressing the CD8 antigen.
CliniMACS CD14 Reagent	Reagent for magnetic labeling of cells expressing the CD14 antigen.
CliniMACS CD19 Reagent	Reagent for magnetic labeling of cells expressing the CD19 antigen.
CliniMACS CD25 Reagent	Reagent for magnetic labeling of cells expressing the CD25 antigen.
CliniMACS CD34 Reagent	Reagent for magnetic labeling of cells expressing the CD34 antigen.
CliniMACS CD45RA Reagent	Reagent for magnetic labeling of cells expressing the CD45RA antigen.
CliniMACS CD56 Reagent	Reagent for magnetic labeling of cells expressing the CD56 antigen.
CliniMACS CD62L Reagent	Reagent for magnetic labeling of cells expressing the CD62L antigen.
CliniMACS CD133 Reagent	Reagent for magnetic labeling of cells expressing the CD133 antigen.
CliniMACS CD271 Reagent	Reagent for magnetic labeling of cells expressing the CD271 antigen.
CliniMACS Cytokine Capture System (IFN-gamma)	Combination of reagents, consisting of the CliniMACS IFN-gamma Catchmatrix Reagent and the CliniMACS IFN-gamma Enrichment Reagent. With the CliniMACS Cytokine Capture System (IFN-gamma), IFN-gamma secreting cells can be enriched after *in vitro* restimulation.
CliniMACS Depletion Tubing Set	Set of tubes, connectors, columns, and bags through which the magnetically labeled cell suspension is processed and in which the magnetic cell separation takes place, especially designed for specific depletion needs.
CliniMACS IFN-gamma Catchmatrix Reagent	Component of the CliniMACS Cytokine Capture System (IFN-gamma) for surface labeling of leucocytes with IFN-gamma specific antibodies.
CliniMACS IFN-gamma Enrichment Reagent	Component of the CliniMACS Cytokine Capture System (IFN-gamma) for magnetic labeling of IFN-gamma bound to the CliniMACS IFN-gamma Catchmatrix Reagent on the IFN-gamma secreting cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS PBS/EDTA Buffer	Buffer used for cell preparation and cell separation with the CliniMACS Plus System: PBS (phosphate buffered saline), supplemented with 1 mM EDTA, pH 7.2. Before use, CliniMACS PBS/EDTA Buffer must be supplemented with pharmaceutical grade HSA to a final concentration of 0.5% (weight/volume, i.e. 5 g HSA per liter buffer).
CliniMACS ^{plus} Instrument	Magnetic cell separation instrument based on the MACS® Technology.
CliniMACS TCRα/β-Biotin	Biotinylated antibody for labeling of human cells expressing the TCRα/β antigen prior to the magnetic labeling with the CliniMACS Anti-Biotin Reagent.
CliniMACS Tubing Set TS, CliniMACS Tubing Set LS	Set of tubes, connectors, columns, and bags through which the magnetically labeled cell suspension is processed and in which the magnetic cell separation takes place.
EDTA	Ethylene-diamine-tetra-acetic acid.
×g	Multiples of the earth's gravitational acceleration.
Heat sealer	Heating device used to sterile seal PVC tubing.
Hematopoietic progenitor cells	Progenitor cells of lymphoid, myeloid, and erythroid lineages.
HSA	Human serum albumin. FDA-licensed HSA is necessary as a buffer supplement when used with the CliniMACS Plus System.
IgG	Immunoglobulin G. An antibody isotype necessary for use with some procedures using the CliniMACS Plus System (e.g. CliniMACS Plus CD3 System).
Labeling	Reaction of cells with magnetic labeling reagent, e.g. CliniMACS CD34 Reagent to CD34 positive cells.
Leukapheresis	Apheresis collecting leukocytes.
Liquid sensor	Component of the CliniMACS ^{plus} Instrument that detects liquid in the tubing.
Luer connector	Screw coupling, part of the tubing set.
MACS®	MACS is a registered trademark of Miltenyi Biotec GmbH.
Magnetic antibody	A super-paramagnetically labeled antibody.
Monoclonal antibodies	A single type of antibody that is directed against a specific epitope (antigen, antigenic determinant) and is produced by a single clone of B cells or a single hybridoma cell line, which is formed by the fusion of a lymphocyte cell with a myeloma cell. Some myeloma cells synthesize single antibodies naturally.
Negative Fraction Bag	Bag of the CliniMACS Tubing Set TS and CliniMACS Tubing Set LS containing the non-target cell fraction.

Non-Target Cell Bag	Bag of the CliniMACS Depletion Tubing Set containing the non-target cell fraction.
Orbital rotator	Device used to mix leukapheresis product during the reaction with CliniMACS Reagents.
Peristaltic pump	Tubing pump. Pump used in the CliniMACS plus Instrument to control the flow rate of fluid in the tubing set.
Plasma extractor	Device used to extract liquid from the Cell Preparation Bag after cell washing.
Plasma Waste Bag	Waste bag to collect excess plasma prior to the labeling procedure.
Pre-column	First column in the CliniMACS Tubing Set TS and the CliniMACS Tubing Set LS, serves as filter to trap cells having non-specific interactions with the column matrix.
Pre-column holder	Support mounted on the CliniMACS plus Instrument that holds the pre-column in place.
Pre-system filter	40 µm filter device between Cell Preparation Bag and pre-column used to trap clumps and cell debris.
Priming	Step prior to cell separation in which buffer is flushed through the tubing set.
Priming Waste Bag	Bag in which buffer from priming step is collected.
Pump safety switch	Sensor that prevents pump operation when the pump door is open.
Reapplication Bag	Bag of the CliniMACS Depletion Tubing Set in which the unlabeled cells are collected temporarily during the separation. The unlabeled cells from the Reapplication Bag are applicated onto the separation column twice, to ensure high purity of the target cells.
Retaining ring	Part of a tubing set that enables the pump tubing to remain in its proper location.
rpm	Revolutions per minute.
Sampling Site Coupler	Injection port, e.g. for removal of samples or addition of CliniMACS Reagents to the Cell Preparation Bag.
Selection buffer	See CliniMACS PBS/EDTA Buffer.
Selection column	See separation column.
Selection column holder	See separation column holder.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Separation column	Column in which magnetically labeled cells are separated when exposed to the magnetic field.
Separation column holder	Moulded guides in the magnet housing that holds the separation column in place.
Separation program	Software program designed for the enrichment or depletion of magnetically labeled cell subsets from a mixed cell population. The operator can choose from a menu of separation programs depending on the intended separation.
Separation reagent	Reagent for magnetic labeling of cells, e.g. CliniMACS CD34 Reagent.
T-fitting	T-shaped fitting on a tubing set where three tubes meet.
TCRα/β antigen	The TCRα/β is the T cell receptor heterodimer composed of two transmembrane glycoprotein chains, α and β. Both chains are members of the Ig superfamily and consist of a constant and a polymorphic variable region. The variable region of the TCRα/β is involved in recognition of antigenic peptides presented by the MHC complex of antigen presenting cells. The TCRα/β-antigen is expressed on the majority of peripheral blood T cells.
Transfer Bag	Bag with a tubing and a spike at the end.
Wash Waste Bag	Collection bag in which the wash supernatant is collected by separation from the sedimented cell suspension after centrifugation steps during sample preparation.
WBC	White blood cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

3 Customer information

3.1 MACS® Magnetic Cell Separation

MACS® Magnetic Cell Sorting is a well proven powerful tool for the separation of many cell types in research laboratories as well as in clinical applications. Cell mixtures can be separated in a magnetic field using an immunomagnetic label specific for the cell type of interest, e.g. human CD34 positive hematopoietic progenitor cells from heterogeneous hematologic cell populations.

3.2 The CliniMACS® Plus System

The CliniMACS® Plus System consists of a computer controlled instrument (CliniMACS ^{plus} Instrument), sterile, magnetic labeling reagents (CliniMACS Reagents), sterile tubing sets (CliniMACS Tubing Sets), and sterile buffer (CliniMACS PBS/EDTA Buffer).

Use of the CliniMACS Plus Reagent Systems are limited by Federal Law to Investigational Use only. In the US, the CliniMACS Plus Systems are available for use only under an approved Investigational New Drug (IND) application or Investigational Device Exemption (IDE).

For information, warnings, and precautions concerning the the components of the CliniMACS Plus System, please refer to the respective chapters of this user manual, e.g., to chapter 1 for information regarding the CliniMACS ^{plus} Instrument or find instructions for use of the CliniMACS Reagents in chapter 4, STEP 1 of the respective application.

3.3 CliniMACS® Reagents and Biotin Conjugates

The CliniMACS® Reagents are dark colored, non-viscous, colloidal solutions, containing the cell specific antibody conjugates in buffer. The conjugates consist of the antibody chemically coupled to super-paramagnetic particles.

The CliniMACS Biotin Conjugates are clear and colorless solutions containing antibody covalently linked to biotin in buffer.

Note

For warnings and precautions concerning CliniMACS Reagents and Biotin, please refer to the respective section in chapter “Four STEPS to your target cells“, STEP 1.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

The antibodies are highly specific, making labeling of rare target cells possible.

CliniMACS Reagents and Biotin Conjugates do not contain and are not derived from any animal orgin known or being suspected of bearing the risk of Transmissible Spongiform Encephalopathies (TSE).

3.4 CliniMACS® Tubing Sets

Intended use

The CliniMACS® Tubing Sets intended for *in vitro* enrichment or depletion of human cells from heterogeneous haematologic cell populations in combination with a CliniMACS plus Instrument, CliniMACS Reagents, and CliniMACS PBS/EDTA Buffer.

Precautions

- Aseptic working procedures must be applied for the unpacking, assembly and use of the tubing set.
- Before human applications, the suitability of the target cells must be demonstrated regarding indication, quality and quantity.
- For the manufacturing and use of target cells in humans, the national legislations and regulations must be followed.
- Any clinical application of the separated cells is exclusively within the responsibility of the user.
- The separation of cells using the tubing sets must be performed by trained operators only.
- All materials which have come into contact with blood and blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.
- Do not connect directly to a patient.
- Do not store blood, blood fractions or cell fractions in the tubing set.
- For additional safety, the integrity test must be performed as described in STEP 3.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings

- Prior to opening the tray holding a tubing set, inspect the package for damage, punctures or tears. Damaged packaging could indicate that the tubing set is no longer sterile and therefore must not be used. Do not use if any leaks are observed during priming or separations.

– Do not use the CliniMACS Tubing Sets after the use-by date printed on the tray label (see fig. 3.1).

Fig. 3.1: Use-by date.

– Do not use the CliniMACS Tubing Sets if package is damaged (see fig. 3.2). Use undamaged and sealed packages only.

Fig. 3.2: Do not use if package is damaged.

- CliniMACS Tubing Sets are for single use only (see fig. 3.3). The reuse of tubings sets or parts of them may lead to the endangerment of the patient due to biological contamination and to inefficient cell separation.

Fig. 3.3: Do not reuse.

– Some components of the device contain the phthalate di(2-ethylhexyl)phthalate (DEHP) as softener (see fig. 3.4). The tubing sets do not release critical concentrations of the plasticizer DEHP. No risk caused by DEHP was identified including children (pre- and postnatal).

Fig. 3.4: Contains phthalates: di(2-ethylhexyl) phthalate (DEHP).

- Store at room temperature.

Further information

– CliniMACS Tubing Sets are sterilized with ethylene oxide (EO) (see fig. 3.5).

Fig. 3.5: Sterile. Method of sterilization using ethylene oxide.

– The tubing sets contain a non-pyrogenic, sterile fluid path (see fig. 3.6).

Fig. 3.6: Non-pyrogenic, sterile fluid path

3.5 CliniMACS® PBS/EDTA Buffer

Important

- Before use, CliniMACS PBS/EDTA Buffer must be supplemented with HSA to a final concentration of 0.5% (w/v). Note that HSA is not a component of the CliniMACS System. Only FDA-licensed HSA should be used. Carefully read the package insert of the HSA used; in particular the section regarding hypersensitivity reactions and the risk of infection that HSA as a blood-derived product brings to all patients. All risks arising from these materials must be evaluated by the user.
- Prior to target cell infusion the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Fig. 3.7: Sterile. Method of sterilization using steam (autoclaving) or dry heat.

Intended purpose

The CliniMACS® PBS/EDTA Buffer is intended for the *in vitro* separation of human cells in combination with the CliniMACS Plus System.

Precautions

- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively within the responsibility of the user.
- The separation of human cells using the CliniMACS Plus System components must be performed by trained operators only.
- All materials which have come into contact with blood and blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- For *in vitro* use only. Not for use in any other cell processing, therapeutic or diagnostic application. Not for parenteral application. Do not infuse into patients.
- Drugs may be incompatible with the buffer. Do not add any drugs to the buffer other than Human Serum Albumin as specified in this User Manual at any time during the separation procedure.

Further information

- The CliniMACS PBS/EDTA Buffer is tested for endotoxins.
- Do not use the CliniMACS PBS/EDTA Buffer after the use-by date printed on the bag label (see fig. 3.1).
- Do not use the CliniMACS PBS/EDTA Buffer if package is damaged (see fig. 3.2). Use undamaged and sealed bags only.
- CliniMACS PBS/EDTA Buffer has been sterilized by autoclaving (see fig. 3.7).
- Store at room temperature. Do not freeze the CliniMACS PBS/EDTA Buffer.

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

3.6 Additional materials required

In addition to the CliniMACS Products, additional materials may be required for a CliniMACS Plus Separation.

– **Transfer bags, suitable for centrifugation**
Transfer Bag 150 mL
Transfer Bag 600 mL
Transfer Bag 1000 mL
Transfer Bag 400 mL, Charter Medical, REF T3104, or equivalent.

- **Cell culture bags, gas-permeable**
Cell Differentiation Bag 100 mL, Miltenyi Biotec, REF 170-076-400, or equivalent.

- **Sampling site coupler**

- **Plasma transfer set**
Transfer Set Coupler/Coupler, Fenwal, or equivalent.

- **Luer/Spike Interconnector**

- **Pre-system filter**
Pre-system Filter, Blood Transfusion Filter, Pall, Ref. No. SQ40S or equivalent.

- **200 µm in-line blood filter**
Fenwal, Product Code 4C2160 or equivalent.

- **Locking forceps or slide clamps**

- **Syringes and needles**
Appropriate syringes (1 mL, 10 mL, 20 mL, 50 mL) and hypodermic 20 gauge needles.

- **Sample tubes**

- **Human serum albumin (HSA)**
Only FDA-licensed HSA should be used.

- **Pharmaceutical grade immunoglobulin G**
[1] Gamunex® 10%, or equivalent.

Note

For information regarding the amount of materials required for an application or further specific materials, please refer to the instructions of the respective application given in chapter "Four STEPS to your target cells" (STEP 1).

1 GAMUNEX® is a trademark of Talecris Biotherapeutics, Inc., USA.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

- **Application specific materials:**
  Individual biotinylated cell specific antibody or ligand; AB serum or, alternatively, autologous serum; cell culture medium.

- **Cell culture medium**
  e.g. RPMI 1640, Miltenyi Biotec, REF 130-091-439, or equivalent.

3.7 Equipment required

- **Uninterruptable power source** (reliable, noise free utility), rated at a minimum of 180 VA. Recommended UPS: APC Smart-UPS 1500VA USB & Serial 120 V, manufactured by APC (American Power Conversion) or equivalent.

- **Laminar flow hood.**

- **Sterile tubing connector**
  Terumo Sterile Connection Device, [2] TSCD® SC-201, or equivalent.

- **Orbital rotator**
  Lab-Line, Model 4635, or equivalent.

- **Centrifuge**
  Sorvall, Model RC3, or equivalent and buckets for centrifugation with aerosol containment caps.

- **Plasma extractor**
  Terumo Equipment, Plasma Separation Stand, Ref. No. 1ME*ACS201, or equivalent.

- **Table top balance**
  Mettler Toledo, Ref. No. 11274-998, or equivalent with 1 kg capacity; resolution to 0.1 g.

- **Tubing heat sealer**
  Baxter, Hematron III, Ref. No. FDR4360, or equivalent.

- **Tubing stripper**
  Baxter, Ref. No. RAR4415, or equivalent.

- **Biohazard waste containers.**

2 TSCD® is a registered trademark of Terumo Corporation, Tokyo, Japan.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Additional equipment required for an application using the CliniMACS Plus Cytokine Capture System (IFN-gamma):

- **24 cell culture dish**
  [3] Corning® Costar® 24 cell culture plate, Sigma Aldrich, Ref. N° CLS3526, or equivalent.

- **MiniMACS Separation Unit**, Miltenyi Biotec, REF 130-042-102.

- **MS Columns**, Miltenyi Biotec, REF 130-042-201.

- **Pre-Separation Filter**, Miltenyi Biotec, REF 130-041-407.

- **MACSmix device**
  MACSmix Tube Rotator, Miltenyi Biotec, REF 130-090-753.

3.8 General warnings and precautions

Separation procedure

1. All separation procedures must be performed by trained operators only. The operator training will be provided by our Miltenyi Biotec authorized personnel.

2. Before human applications, the suitability of the target cells must be demonstrated regarding indication, quality and quantity.

3. Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.

4. Any clinical application of the separated cells is exclusively within the responsibility of the user.

5. All blood products and materials which have come into contact with blood and blood products, must be treated as infectious material. Regulations for the handling of infectious material must be observed.

6. All cell preparation and labeling procedures must be performed at room temperature (+19°C to +25°C) unless otherwise stated. Higher ambient temperature results in less purity and yield of the target cells.

Limited warranty

Should the CliniMACS Plus System be used in a manner not explicitly described in this manual, all warranties will be null and void.

3 Corning® and Costar® are registered trademarks of Corning Incorporated, Corning, NY, U.S.A.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

7. All tubing, fittings, valves, the pre-column, and the separation column should be checked thoroughly for leaks during the priming step.

8. All bags, including those used in sample preparation, should be preserved until final analysis of the collected cells has been completed and successful separation of the target cells has been confirmed.

Handling of biohazardous material

- To avoid contamination of the cellular starting product, all preparation steps should be performed using aseptic techniques.

- The operator performing the cell separation must be trained in the proper use of the equipment and in the handling of blood products and bone marrow aspirate.

- The operator performing the cell separation should wear appropriate clothing (e.g. lab coat, gloves and eye glasses or goggles) when working with a patient sample and handling potentially biohazardous material.

- All blood products must be treated as a potential biohazard. Leukapheresis product, blood product, bone marrow aspirate, collected cells, used buffer, used tubing set and other materials that have been in contact with these fluids must be treated as biohazardous materials according to standard hospital or institutional requirements.

- The CliniMACS plus Instrument should be considered a potential biohazard after each separation run and cleaned with an aqueous biocidal detergent (e.g. Bacillol® plus or Meliseptol®, see also chapter 1 “Cleaning and maintenance of the CliniMACS plus Instrument“) according to standard hospital or institutional requirements.

- Disposable materials should be treated according to standard hospital or institutional requirements for biohazardous materials.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Cellular starting product

- The cellular starting product (e.g. leukapheresis product, buffycoat, bone marrow aspirate) should be collected according to standard hospital or institutional procedures in standard collection bags. Bone marrow aspirate should be collected in heparin-coated containers (e.g. 5 mL syringes). Labeling and separation of cells should begin as soon as possible after the cellular product has been collected. Prior to the cell labeling procedure, no additional anticoagulants or blood additives (heparin, etc.) should be included beyond those normally used during leukapheresis or during bone marrow aspiration.

- The container containing the cellular starting product should be labeled with patient identification, time, date and place of collection according to procedures specified for use with the clinical protocol.

- For transportation of the leukapheresis product or buffy coat the product should be packed in insulated containers and should be kept at controlled room temperature (+19 °C to +25 °C) according to standard hospital or institutional blood collection procedures approved for use with the clinical protocol. Do not refrigerate. The cell concentration should not exceed 0.2×10^9 cells per mL during transportation.

- For transportation of the bone marrow aspirate, the product should be packed in an insulated container and should be kept at controlled temperature (+4 °C).

- Avoid intensive mixing of the cellular starting material.

- If the cellular starting product must be stored, e.g. overnight, it should be kept at controlled room temperature (+19 °C to +25 °C). Bone marrow aspirate should be kept at controlled temperature (+4 °C). During storage, the concentration of leukocytes should never exceed 0.2×10^9 cells per mL.

- Cells should be stored in autologous plasma. If the cell concentration is higher than 0.2×10^9 cells per mL, dilute the product with autologous plasma.

Important

Labeling and separation of cells should begin as soon as possible after the cellular starting product has been collected. The product should not be older than 24 hours when starting the labeling and separation procedure.

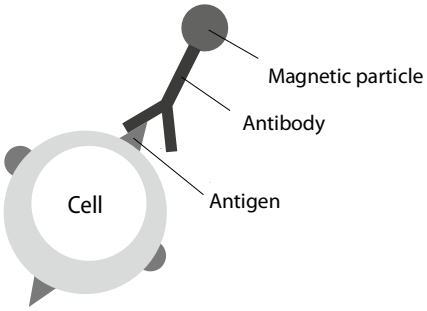


Fig. 3.8: Magnetic labeling of cells.

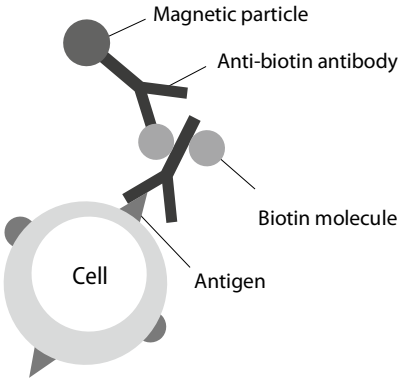


Fig. 3.9: Flexible Labeling System.

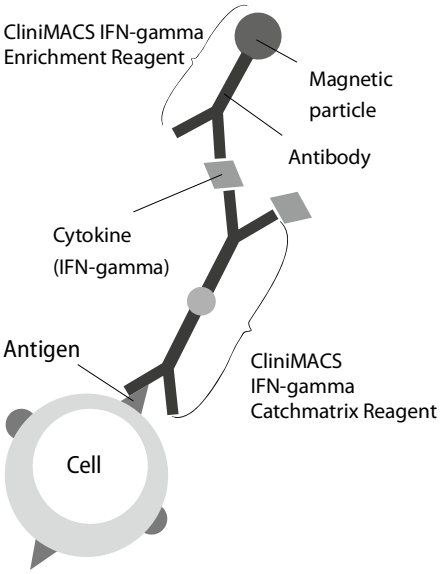


Fig. 3.10: CliniMACS Cytokine Capture System (IFN-gamma).

3.9 Labeling of cells with CliniMACS® Reagents

Using the CliniMACS® Plus System cells can be magnetically labeled in three different ways:

- Directly in a one-way labeling procedure by antigen-specific antibodies conjugated to super-paramagnetic iron-dextran beads (see fig. 3.8).

- Indirectly in a two-step labeling procedure called the Flexible Labeling System. In a first step cells are labeled with antigen-specific antibodies conjugated to biotin. In a second step these antibodies are labeled with biotin-specific antibodies conjugated to super-paramagnetic iron-dextran beads (see fig. 3.9).

- Indirectly in a two-step labeling procedure called the CliniMACS Plus Cytokine Capture System (IFN-gamma). First, cells are *in vitro* restimulated to secrete the cytokine (e.g. IFN-gamma). In a first labeling step the restimulated cells are labeled by a hybrid-molecule consisting of a leucocyte-specific antibody and a cytokine-specific antibody, called the CliniMACS IFN-gamma Catchmatrix Reagent. The cells are then incubated to allow secretion of the cytokine, which is "captured" by the catchmatrix on the cell surface. Subsequently, in a second labeling step the cytokine secreting cells are labeled with cytokine-specific antibodies conjugated to super-paramagnetic iron-dextran beads (CliniMACS IFN-gamma Enrichment Reagent), (see fig. 3.10).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

3.10 High-gradient magnetic cell separation

The magnetically labeled cell suspension is loaded onto the CliniMACS plus Instrument prepared with a tubing set. This high-gradient magnetic cell separation unit consists of a specifically developed, powerful permanent magnet and a separation column with a ferromagnetic matrix.

The high-gradient field allows the generation of strong magnetic forces and a rapid demagnetization. When small ferromagnetic structures, such as the column matrix, are placed within the magnetic field they disrupt the homogeneity of the field. This results in the generation of high magnetic gradients. In their immediate surrounding the ferromagnetic structures generate magnetic forces 10,000-fold stronger than in conventional geometries. The high-gradient field attracts labeled cells to the matrix and effectively retains them. After removing the column from the magnet, the rapid demagnetization of the column matrix allows the release of retained cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

3.11 CliniMACS® Separation strategies

The CliniMACS® Plus System provides the user with a variety of separation programs. The separation programs can generally be divided into enrichment strategies (CD34 SELECTION 1 and 2, CD133 SELECTION 1 and 2, ENRICHMENT 1.1, and ENRICHMENT 3.2) and depletion strategies (DEPLETION 2.1 and DEPLETION 3.1).

Enrichment of magnetically labeled cells

When choosing an enrichment strategy, the magnetically labeled cells (primary labeled with a CliniMACS Reagent) are retained in the separation column and the non-labeled cells pass through. The **labeled cells** (target cells) are collected in the **Cell Collection Bag** and the **non-labeled cells** (non-target cells) in the **Negative Fraction Bag** (see fig. 3.11).

Depletion of magnetically labeled cells

The MACS Technology is also very efficient for depleting specific cell populations. Unwanted cells are specifically labeled with super-paramagnetic particles and separated from target cells upon passage through the high-gradient magnetic column. In contrast to the enrichment strategy, the **non-labeled cells** (target cells) are collected **in the Cell Collection Bag** and the **labeled cells** (non-target cells) are collected in the **Negative Fraction Bag** (see fig. 3.12) or in the **Non-Target Cell Bag** (see fig. 3.13 and fig 3.14) respectively.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment strategy

Enrichment of cells using the CliniMACS Tubing Set TS or CliniMACS Tubing Set LS

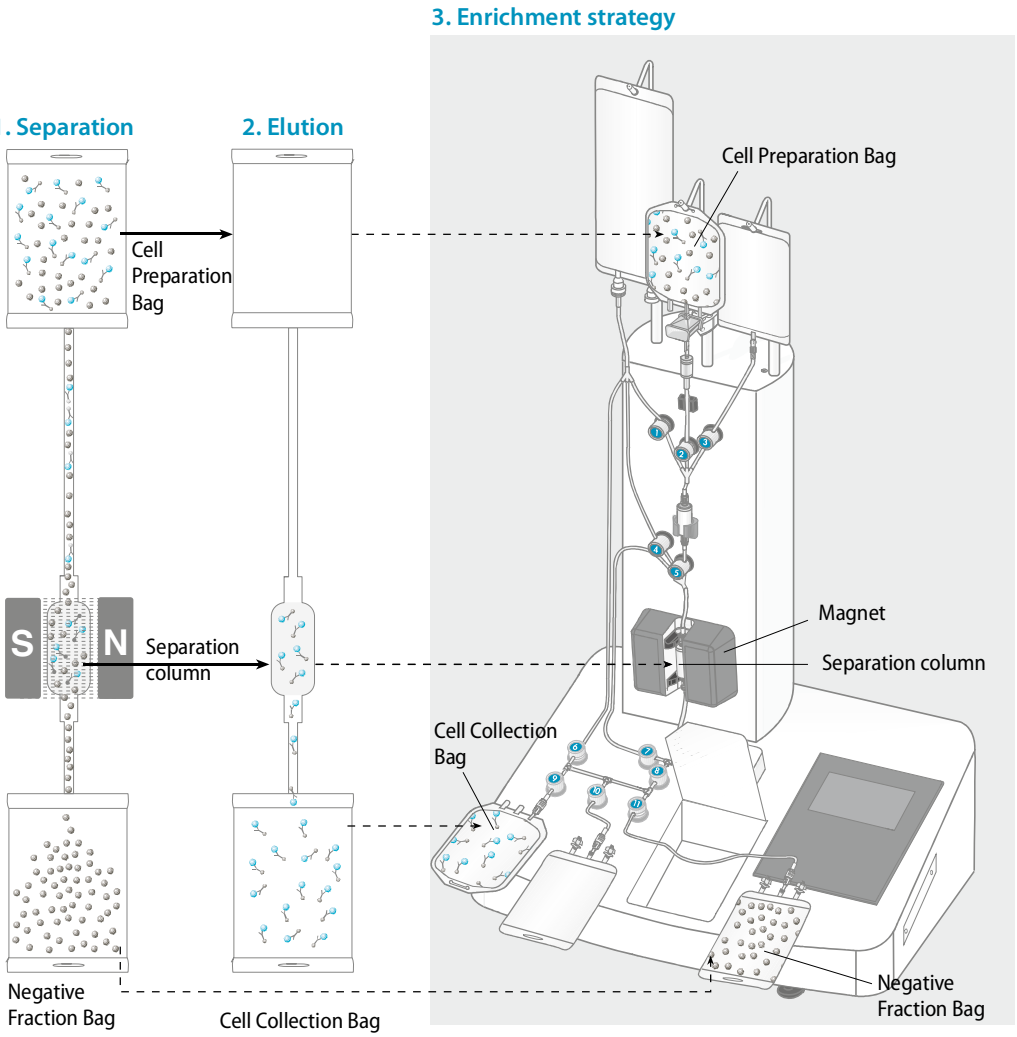


Fig. 3.11: Strategy of enrichment programs using the CliniMACS Tubing Set TS or CliniMACS Tubing Set LS.

1. The magnet is in the "ON"-position. Magnetically labeled cells are held in the separation column, while other non-labeled cells (non-target cells) flow through the column and are collected in the Negative Fraction Bag.

2. The magnet is in the "OFF"-position. The magnetically labeled cells (target cells) are released from the separation column and collected in the Cell Collection Bag.

3. The **enrichment program** retains the magnetically labeled cells in the separation column, the **non-labeled cells** (non-target cells) flow through the column and are collected in the **Negative Fraction Bag**. When the magnet is moved into the "OFF"-position, the **magnetically labeled cells** (target cells) are released from the column and collected in the **Cell Collection Bag**.

Note

The target cell fraction is always collected in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Depletion strategy

Depletion of cells using the CliniMACS Tubing Set LS - DEPLETION 2.1

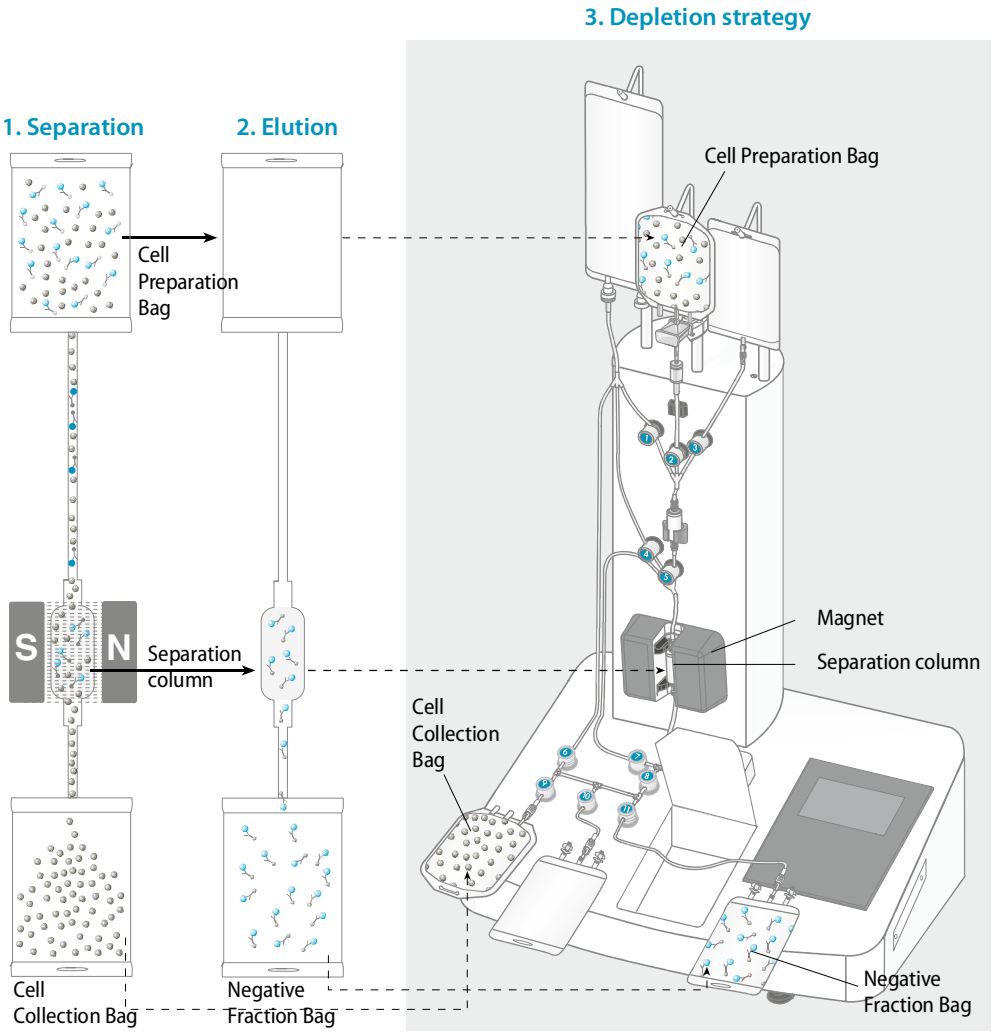


Fig. 3.12: Strategy of the depletion program DEPLETION 2.1 using the CliniMACS Tubing Set LS.

1. The magnet is in the "ON"-position. Magnetically labeled cells are held in the separation column, while other non-labeled cells (target cells) flow through the column and are collected in the Cell Collection Bag.

2. The magnet is in the "OFF"-position. The magnetically labeled cells (non-target cells) are released from the separation column and collected in the Negative Fraction Bag.

3. The **depletion program** (DEPLETION 2.1) retains the magnetically labeled cells in the separation column, the **non-labeled target cells** (target cells) flow through and are collected in the **Cell Collection Bag**. When the magnet is moved into the "OFF"-position, the **magnetically labeled cells** (non-target cells) are released from the column and collected in the **Negative Fraction Bag**.

Note

The target cell fraction is always collected in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Depletion of cells using the CliniMACS Depletion Tubing Set - DEPLETION 3.1

(Part 1: BULK LOADING STAGE)

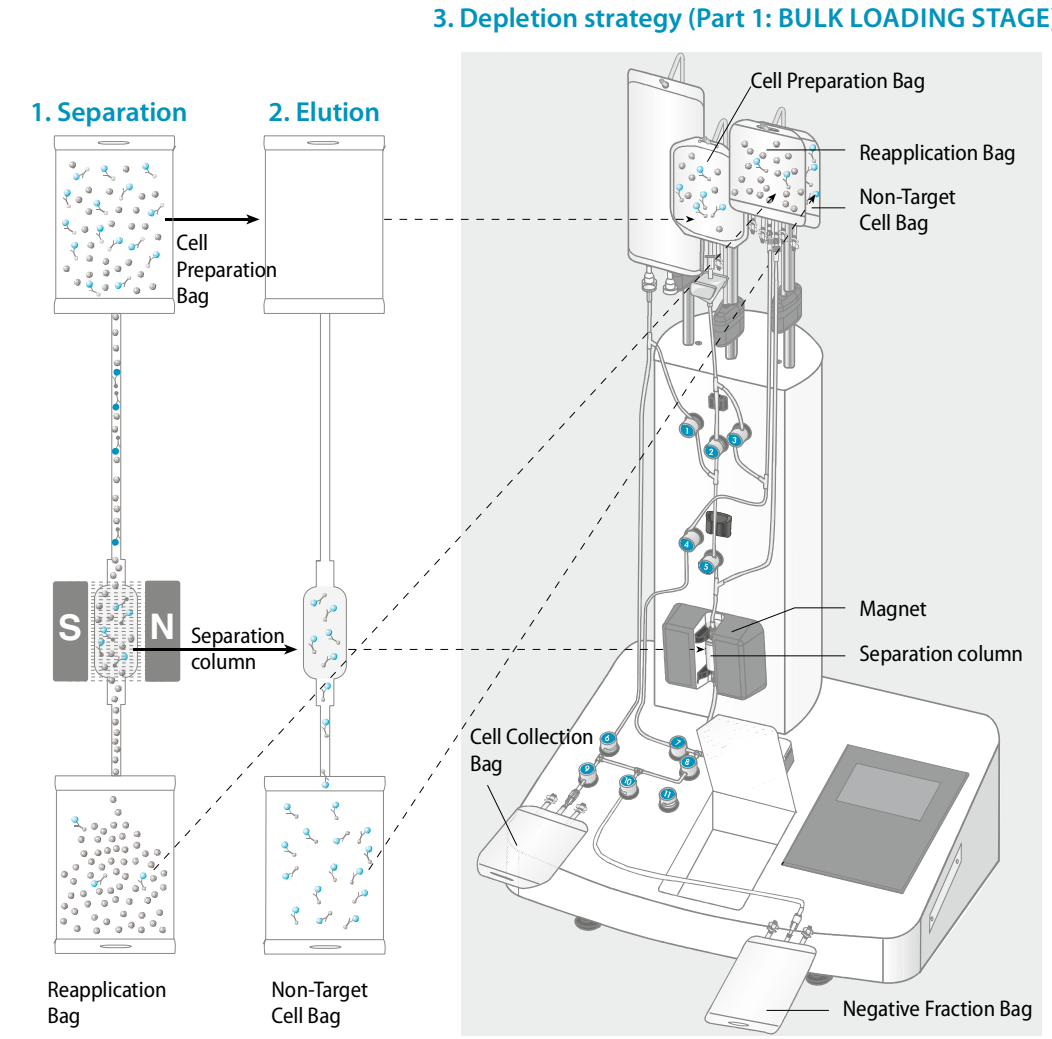


Fig. 3.13: Strategy of the depletion program DEPLETION 3.1 using the CliniMACS Depletion Tubing Set (Part 1: BULK LOADING STAGE).

1. BULK LOADING STAGE: The magnet is in the "ON"-position. Magnetically labeled cells are held in the separation column, while other non-labeled cells (target cells) flow through and are collected in the Reapplication Bag.

2. The magnet is in the "OFF"-position. The magnetically labeled cells (non-target ccells) are released from the separation column and collected in the Non-Target Cell Bag.

3. The **depletion program** (DEPLETION 3.1) retains the magnetically labeled cells in the separation column, the **non-labeled target cells** (target cells) flow through the column and are collected in the **Reapplication Bag** in order to be reloaded onto the separation column in a second SENSITIVE LOADING STAGE (see fig. 3.14). When the magnet is moved into the "OFF"-position, the **magnetically labeled cells** (non-target cells) are released from the column and collected in the **Non-Target Cell Bag**.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Depletion of cells using the CliniMACS Depletion Tubing Set - DEPLETION 3.1

(Part 2: SENSITIVE LOADING STAGE)

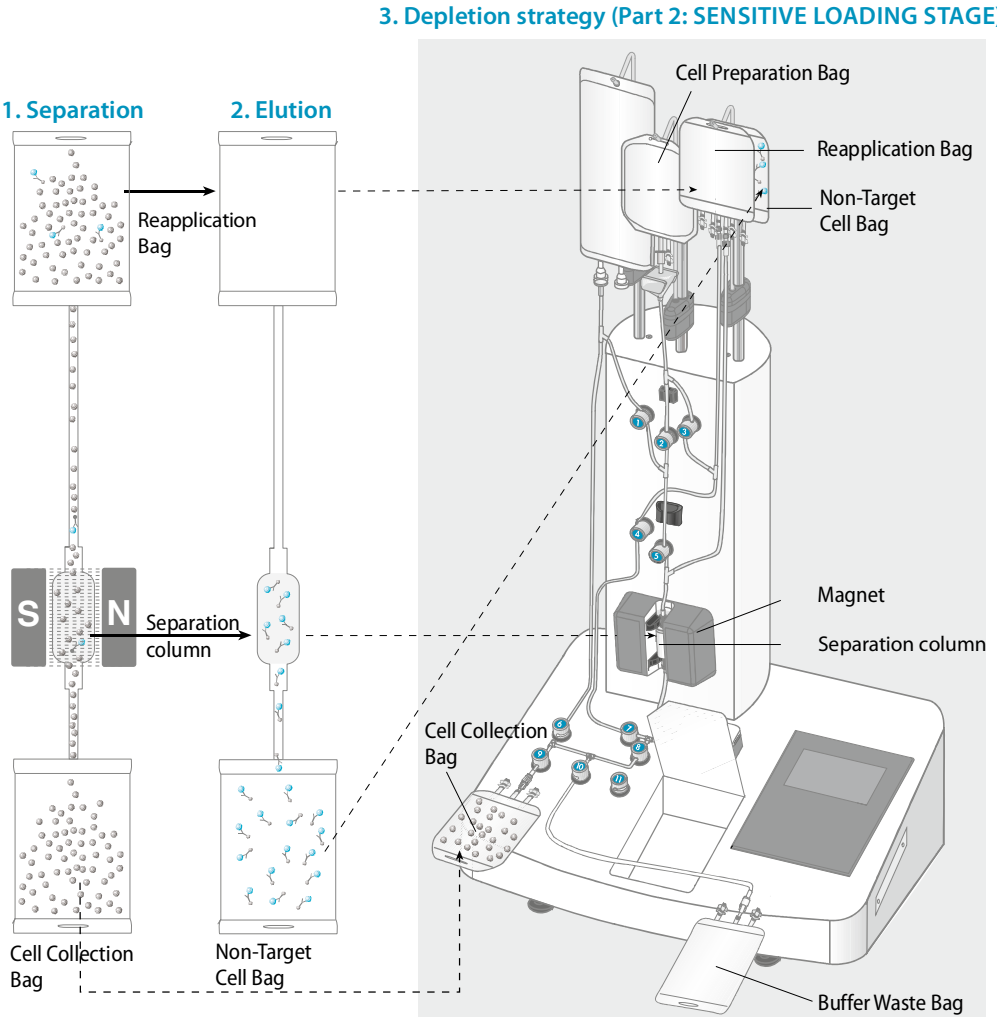


Fig. 3.14: Strategy of the depletion program DEPLETION 3.1 using the CliniMACS Depletion Tubing Set (Part 2: SENSITIVE LOADING STAGE).

4. SENSITIVE LOADING STAGE: The magnet is in the "ON"-position. The cells from the Reapplication Bag are reloaded on the separation column. The few remaining magnetically labeled cells are held in the separation column, while the non-labeled cells (target cells) flow through the column and are collected in the Cell Collection Bag.

5. Then the magnet is moved to the "OFF"-position. The magnetically labeled cells (non-target cells) are released from the separation column and collected in the Non-Target Cell Bag.

6. At the end of the separation the **labeled cells** (non-target cells) are in the **Non-Target Cell Bag** and the **unlabeled cells** (target cells) are **in the Cell Collection Bag**.

Note

The target cell fraction is always collected in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

4 Four STEPS to your target cells

In the following chapter an overview of the CliniMACS Plus Separation is presented. The CliniMACS Plus Separation is carried out in Four STEPS and the User Manual is divided into sections to describe each step. Before starting the separation, the applicable chapters of the User Manual must be chosen by the operator by using the overview table beginning on page "Four STEPS -Start-2". This table summarizes information regarding specific chapters based on the type and amount of reagents used, the number of cells, the type of tubing set utilized and the relevant separation program required. For the applications, worksheets may be provided to record important information and data generated during sample preparation and separation. If the worksheet of the application to be performed is not part of this manual, please contact the Miltenyi Biotec Technical Support.

STEP 1 - Located in the Green Section of the CliniMACS User Manual

STEP 1 describes the preparation of the cell product and the magnetic labeling of the specific cells, expressing the respective antigen. Follow the instructions of the applicable section (e.g. for enrichment of CD133 positive cells, proceed to section "CliniMACS CD133 Reagent"). Continue with STEP 2.

STEP 2 - Located in the Orange Section of the CliniMACS User Manual

STEP 2 describes the selection of a separation program available using the CliniMACS plus Instrument. Depending on cell type and cell number to be separated, different programs must be selected. To determine which program is applicable, go to the overview table on page "Four STEPS-Start-2". Read the chapter indicated for the selected separation program (e.g. for CD133 normal scale enrichment the applicable separation program is CD133 SELECTION 1). Follow the instructions given in the applicable section. Continue with STEP 3.

STEP 3 - Located in the Brown Section of the CliniMACS User Manual

STEP 3 explains the installation of the tubing set onto the CliniMACS plus Instrument. Follow the instructions in the applicable chapter ("CliniMACS Tubing Set TS (REF 161-01) and CliniMACS Tubing Set LS (REF 162-01)" or "CliniMACS Depletion Tubing Set (REF 261-01)"). Continue with STEP 4.

STEP 4 - Located in the Red Section of the CliniMACS User Manual

STEP 4 describes the automated CliniMACS Plus Separation. The CliniMACS plus Instrument performs the automated cell separation procedure (chosen in STEP 2). Follow the instructions in the applicable chapter (e.g. for the enrichment of CD14 positive cells, choose chapter "CliniMACS Run - ENRICHMENT 1.1").

Important

- The overview table on the following pages summarizes information on **typical applications** of the CliniMACS Reagents. When separation of a different number of target cells is to be performed, refer to the detailed instructions in STEP 1 to find information on the maximum capacity of the respective application.
- The color coding represents the "Four STEPS". Follow the instructions of the **applicable** chapters. See overview table.

Example of overview table

For the separation of up to 0.6×10^9 CD133 positive cells from 60×10^9 total cells (small scale enrichment) one vial of CliniMACS CD133 Reagent (REF 172-01) and a CliniMACS Tubing Set TS (REF 161-01) are needed. For the procedure follow the subchapters:

› STEP 1: CliniMACS® CD133 Reagent,
› STEP 2: CD133 SELECTION 1/2,
› STEP 3: CliniMACS® Tubing Set TS and CliniMACS Tubing Set LS and
› STEP 4: CD133 SELECTION 1/2.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Antigen/ Reagent	Anti-Biotin REF 173-01	Anti-Biotin REF 173-01	Anti-Biotin REF 173-01	CCS REF 279-01	CD3 REF 273-01
Application	enrichment	depletion	depletion (large scale)	enrichment	depletion
Application capacity	max. 40×10^9 total cells	max. 40×10^9 total cells	max. 80×10^9 total cells	IFN-secreting cells from 1×10^9 total cells	15×10^9 $CD3^+$ cells from 40×10^9 total cells
No. of vials	1	1	2	1 kit	1
Tubing set (TS)	TS (REF 161-01)	TS LS (REF 162-01)	TS LS (REF 162-01)	TS (REF 161-01)	TS LS (REF 162-01)
No. of TS required for application	1	1	1	1	1
Software (Separation program)	ENRICHMENT 1.1	DEPLETION 2.1	DEPLETION 2.1	ENRICHMENT 3.2	DEPLETION 2.1

Applicable sections

STEP 1 Magnetic labeling	CliniMACS® Anti-Biotin Reagent	CliniMACS® Anti-Biotin Reagent	CliniMACS® Anti-Biotin Reagent	CliniMACS® Cytokine Capture System (IFN-gamma)	CliniMACS® CD3 Reagent
STEP 2 Choice of program	ENRICHMENT 1.1	DEPLETION 2.1	DEPLETION 2.1	ENRICHMENT 3.2	DEPLETION 2.1
STEP 3 Installation of tubing set	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS
STEP 4 CliniMACS® Separation	ENRICHMENT 1.1	DEPLETION 2.1	DEPLETION 2.1	ENRICHMENT 3.2	DEPLETION 2.1

Regulatory Statement ■ CAUTION: Limited by Federal (or United States) Law to Investigational Use.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Antigen/ Reagent	CD3 REF 273-01	CD3 REF 273-01	CD3 REF 273-01	CD3/CD56 Combination	
Application	depletion (large scale)	depletion	depletion (large scale)	Part 1: CD3 depletion	Part 2: CD56 enrichment
				depletion	enrichment
Application capacity	20×10^9 $CD3^+$ cells from 80×10^9 total cells	15×10^9 $CD3^+$ cells from 40×10^9 total cells	30×10^9 $CD3^+$ cells from 80×10^9 total cells	15×10^9 $CD3^+$ cells from 40×10^9 total cells	5×10^9 $CD56^+$ cells from 40×10^9 total cells
No. of vials	2	1	2	1	1
Tubing set (TS)	TS LS (REF 162-01)	DTS (REF 261-01)	DTS (REF 261-01)	DTS (REF 261-01)	TS (REF 161-01)
No. of TS required for application	1	1	1	1	1
Software (Separation program)	DEPLETION 2.1	DEPLETION 3.1	DEPLETION 3.1	DEPLETION 3.1	ENRICHMENT 1.1

Applicable sections

STEP 1 Magnetic labeling	CliniMACS® CD3 Reagent	CliniMACS® CD3 Reagent	CliniMACS® CD3 Reagent	CliniMACS® CD3 Reagent	CliniMACS® CD56 Reagent
STEP 2 Choice of program	DEPLETION 2.1	DEPLETION 3.1	DEPLETION 3.1	DEPLETION 3.1	ENRICHMENT 1.1
STEP 3 Installation of tubing set	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Depletion Tubing Set	CliniMACS® Depletion Tubing Set	CliniMACS® Depletion Tubing Set	CliniMACS® Tubing Set and CliniMACS Tubing Set LS
STEP 4 CliniMACS® Separation	DEPLETION 2.1	DEPLETION 3.1	DEPLETION 3.1	DEPLETION 3.1	ENRICHMENT 1.1

Regulatory Statement ■ CAUTION: Limited by Federal (or United States) Law to Investigational Use.

CliniMACS®

Antigen/ Reagent	CD3/CD19 Combination	CD3/CD19 Combination	CD3/CD19 Combination	CD4 REF 276-01	CD4 REF 276-01
Application	depletion	depletion	depletion (large scale)	enrichment	depletion
Application capacity	15×10^9 $CD3^+$ and 5×10^9 $CD19^+$ cells from 40×10^9 total cells	15×10^9 $CD3^+$ and 5×10^9 $CD19^+$ cells from 40×109 total cells	30×10^9 $CD3^+$ and 10×10^9 $CD19^+$ cells from 80×10^9 total cells	5×10^9 $CD4^+$ cells from 40×10^9 total cells	12×10^9 $CD4^+$ cells from 40×10^9 total cells
No. of vials	1 each	1 each	2 each	1	1
Tubing set (TS)	TS LS (REF 162-01)	DTS (REF 261-01)	DTS (REF 261-01)	TS (REF 161-01)	TS LS (REF 162-01)
No. of TS required for application	1	1	1	1	1
Software (Separation program)	DEPLETION 2.1	DEPLETION 3.1	DEPLETION 3.1	ENRICHMENT 1.1	DEPLETION 2.1

Applicable sections

STEP 1 Magnetic labeling	CliniMACS® CD3/CD19 Reagent Combination	CliniMACS® CD3/CD19 Reagent Combination	CliniMACS® CD3/CD19 Reagent Combination	CliniMACS® CD4 Reagent	CliniMACS® CD4 Reagent
STEP 2 Choice of program	DEPLETION 2.1	DEPLETION 3.1	DEPLETION 3.1	ENRICHMENT 1.1	DEPLETION 2.1
STEP 3 Installation of tubing set	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Depletion Tubing Set	CliniMACS® Depletion Tubing Set	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS
STEP 4 CliniMACS® Separation	DEPLETION 2.1	DEPLETION 3.1	DEPLETION 3.1	ENRICHMENT 1.1	DEPLETION 2.1

Regulatory Statement ■ CAUTION: Limited by Federal (or United States) Law to Investigational Use.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Antigen/ Reagent	CD4 REF 276-01	CD8 REF 275-01	CD8 REF 275-01	CD8 REF 275-01	CD8 REF 275-01
Application	depletion (large scale)	enrichment	enrichment (large scale)	depletion	depletion (large scale)
Application capacity	20×10^9 $CD4^+$ cells from 80×10^9 total cells	4×10^9 $CD8^+$ cells from 40×10^9 total cells	5×10^9 $CD8^+$ cells from 80×10^9 total cells	4×10^9 $CD8^+$ cells from 40×10^9 total cells	8×10^9 $CD8^+$ cells from 80×10^9 total cells
No. of vials	2	1	2	1	2
Tubing set (TS)	TS LS (REF 162-01)	TS (REF 161-01)	TSLS (REF 162-01)	TS LS (REF 162-01)	TS LS (REF 162-01)
No. of TS required for application	1	1	1	1	1
Software (Separation program)	DEPLETION 2.1	ENRICHMENT 1.1	ENRICHMENT 1.1	DEPLETION 2.1	DEPLETION 2.1

Applicable sections

STEP 1 Magnetic labeling	CliniMACS® CD4 Reagent	CliniMACS® CD8 Reagent	CliniMACS® CD8 Reagent	CliniMACS® CD8 Reagent	CliniMACS® CD8 Reagent
STEP 2 Choice of program	DEPLETION 2.1	ENRICHMENT 1.1	ENRICHMENT 1.1	DEPLETION 2.1	DEPLETION 2.1
STEP 3 Installation of tubing set	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS
STEP 4 CliniMACS® Separation	DEPLETION 2.1	ENRICHMENT 1.1	ENRICHMENT 1.1	DEPLETION 2.1	DEPLETION 2.1

Regulatory Statement ■ CAUTION: Limited by Federal (or United States) Law to Investigational Use.

CliniMACS®

Antigen/ Reagent	CD14 REF 272-01	CD19 REF 179-01	CD19 REF 179-01	CD25 REF 274-01	CD25 REF 274-01
Application	enrichment	depletion	depletion (large scale)	enrichment $CD25^+$ cells	enrichment CD25 highly expressing cells
Application capacity	4×10^9 $CD14^+$ cells from 20×10^9 total cells	5×10^9 $CD19^+$ cells from 40×10^9 total cells	10×10^9 $CD19^+$ cells from 80×10^9 total cells	0.6×10^9 $CD25^+$ cells from 40×10^9 total cells (WBC)	0.6×10^9 highly expressing $CD25^+$ cells from 40×10^9 total cells (WBC)
No. of vials	1 or 2	1	2	1	1
Tubing set (TS)	TS (REF 161-01)	TS LS (REF 162-01)	TS LS (REF 162-01)	TS (REF 161-01)	TS (REF 161-01)
No. of TS required for application	1	1	1	1	1
Software (Separation program)	ENRICHMENT 1.1	DEPLETION 2.1	DEPLETION 2.1	ENRICHMENT 3.2	ENRICHMENT 3.2

Applicable sections

STEP 1 Magnetic labeling	CliniMACS® CD14 Reagent	CliniMACS® CD19 Reagent	CliniMACS® CD19 Reagent	CliniMACS® CD25 Reagent	CliniMACS® CD25 Reagent
STEP 2 Choice of program	ENRICHMENT 1.1	DEPLETION 2.1	DEPLETION 2.1	ENRICHMENT 3.2	ENRICHMENT 3.2
STEP 3 Installation of tubing set	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS
STEP 4 CliniMACS® Separation	ENRICHMENT 1.1	DEPLETION 2.1	DEPLETION 2.1	ENRICHMENT 3.2	ENRICHMENT 3.2

Regulatory Statement ■ CAUTION: Limited by Federal (or United States) Law to Investigational Use.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Antigen/ Reagent	CD25 REF 274-01	CD25 REF 274-01	CD34 REF 171-01	CD34 REF 171-01	CD45RA REF 701-46
Application	depletion $CD25^+$ cells	depletion $CD25^+$ cells (large scale)	enrichment	enrichment (large scale)	depletion
Application capacity	6×10^9 $CD25^+$ cells from 40×10^9 total cells (WBC)	12×10^9 $CD25^+$ cells from 80×10^9 total cells (WBC)	0.6×10^9 $CD34^+$ cells from 60×10^9 total cells	1.2×10^9 $CD34^+$ cells from 120×10^9 total cells	20×10^9 $CD45RA^+$ cells from 50×10^9 total cells
No. of vials	1	2	1	2	1
Tubing set (TS)	TS LS (REF 162-01)	TS LS (REF 162-01)	TS (REF 161-01)	TS LS (REF 162-01)	DTS (REF 261-01)
No. of TS required for application	1	1	1	1	1
Software (Separation program)	DEPLETION 2.1	DEPLETION 2.1	CD34 SELECTION 1	CD34 SELECTION 2	DEPLETION 3.1

Applicable sections

STEP 1 Magnetic labeling	CliniMACS® CD25 Reagent	CliniMACS® CD25 Reagent	CliniMACS® CD34 Reagent	CliniMACS® CD34 Reagent	CliniMACS® CD45RA Reagent
STEP 2 Choice of program	DEPLETION 2.1	DEPLETION 2.1	CD34 SELECTION 1/2	CD34 SELECTION 1/2	DEPLETION 3.1
STEP 3 Installation of tubing set	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Depletion Tubing Set
STEP 4 CliniMACS® Separation	DEPLETION 2.1	DEPLETION 2.1	CD34 SELECTION 1/2	CD34 SELECTION 1/2	DEPLETION 3.1

Regulatory Statement ■ CAUTION: Limited by Federal (or United States) Law to Investigational Use.

Antigen/ Reagent	CD56 REF 271-01	CD62L REF 701-19	CD133 REF 172-01	CD133 REF 172-01	CD133 REF 172-01
Application	enrichment	enrichment	enrichment	enrichment (large scale)	enrichment (bone marrow)
Application capacity	5×10^9 $CD56^+$ cells from 40×10^9 total cells	4×10^9 $CD62L^+$ cells from 10×10^9 total cells	0.6×10^9 $CD133^+$ cells from 60×10^9 total cells	1.2×10^9 $CD133^+$ cells from 120×10^9 total cells	50 mL aspirate and max. 120×10^9 total cells
No. of vials	1	1	1	2	1 or 2
Tubing set (TS)	TS (REF 161-01)	TS LS (REF 162-01)	TS (REF 161-01)	TS LS (REF 162-01)	TS LS (REF 162-01)
No. of TS required for application	1	1	1	1	1
Software (Separation program)	ENRICHMENT 1.1	ENRICHMENT 1.1	CD133 SELECTION 1	CD133 SELECTION 2	CD133 SELECTION 2

Applicable sections

STEP 1 Magnetic labeling	CliniMACS® CD56 Reagent	CliniMACS® CD62L Reagent	CliniMACS® CD133 Reagent (Leukapheresis product)	CliniMACS® CD133 Reagent (Leukapheresis product)	CliniMACS® CD133 Reagent (Bone marrow)
STEP 2 Choice of program	ENRICHMENT 1.1	ENRICHMENT 1.1	CD133 SELECTION 1/2	CD133 SELECTION 1/2	CD133 SELECTION 1/2
STEP 3 Installation of tubing set	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS
STEP 4 CliniMACS® Separation	ENRICHMENT 1.1	ENRICHMENT 1.1	CD133 SELECTION 1/2	CD133 SELECTION 1/2	CD133 SELECTION 1/2

Regulatory Statement ■ CAUTION: Limited by Federal (or United States) Law to Investigational Use.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Antigen/ Reagent	CD271 REF 701-12	TCRα/ß-Biotin REF 200-070-407
Application	enrichment (bone marrow)	depletion
Application capacity	100 mL aspirate and max. 1×10^7 $CD271^+$ cells	24×10^9 $TCR\alpha/\beta^+$ cells from 60×10^9 total cells
No. of vials	1 or 2	TCRa/b-Biotin Kit: 1 TCRα/β-Biotin 2 Anti-Biotin Reagents
Tubing set (TS)	TS (REF 161-01)	DTS (REF 261-01)
No. of TS required for application	1	1
Software (Separation program)	ENRICHMENT 3.2	DEPLETION 3.1

STEP 1 Magnetic labeling	CliniMACS® CD271 Reagent	CliniMACS® TCRα/β-Biotin
STEP 2 Choice of program	ENRICHMENT 3.2	DEPLETION 3.1
STEP 3 Installation of tubing set	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Depletion Tubing Set
STEP 4 CliniMACS® Separation	ENRICHMENT 3.2	DEPLETION 3.1

Regulatory Statement ■ CAUTION: Limited by Federal (or United States) Law to Investigational Use.

Enrichment of CD34 positive cells

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD34 Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material

Normal scale: Up to 0.6×10^9 positive cells out of 60×10^9 total cells (WBC):
1 vial of CliniMACS CD34 Reagent (REF 171-01), 1 CliniMACS Tubing Set TS (REF 161-01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Large scale: 0.6×10^9 to 1.2×10^9 positive cells out of 120×10^9 total cells (WBC):
2 vials of CliniMACS CD34 Reagent (REF 171-01), 1 CliniMACS Tubing Set LS (REF 162-01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- Label bags as: **Cell Collection**, **Cell Preparation, Plasma Waste**, **Wash Waste No. 1** and **Wash Waste No. 2**.
- Determine the weight of the empty Cell Collection and Cell Preparation Bag.

III. Leukapheresis product (LP)

Analysis of LP

Transfer of LP into Cell Preparation Bag

Dilution of LP 1:3 — Add buffer: Amount of buffer to be added = Weight of LP × 2

Centrifugation — 200×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

Volume adjustment — Labeling volume: **95 g (normal scale application)**
190 g (large scale application)

- Remove supernatant to adjust the sample using the following equation:
  Weight of supernatant to be removed = Weight of diluted LP – Labeling volume
- Resuspend the cell pellet.

IV. Magnetic labeling of the cells

Incubation with CliniMACS® CD34 Reagent

Add 1 or 2 vials of CliniMACS CD34 Reagent, incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent:

- Fill Cell Preparation Bag with buffer.
- Centrifuge (200×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Fill Cell Preparation Bag with buffer.
- Centrifuge (200×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Adjust volume: **Normal scale**: 150 g
  Large scale: 275 g

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment of CD34 positive cells

Four STEPS to your target cells

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program CD34 SELECTION 1/2

(using 1 vial : CD34 SELECTION 1, or using 2 vials: CD34 SELECTION 2)

STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Cell Collection Bag

Attach Priming Waste Bag and insert pre-column

Insert separation column and load valve no. 5

Load valves nos. 1, 2, 3, and 4

Load pump tubing

Load valves nos. 7 and 8

Load valves nos. 6, 9, 10, and 11

Recheck all tubing and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment of CD133 positive cells

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD133 Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material

- **Normal scale application:** 0.6×10^9 CD133 positive cells out of 60×10^9 total cells (WBC): **1 vial** of CliniMACS CD133 Reagent (REF 172-01), CliniMACS Tubing Set TS (REF 161-01), CliniMACS PBS/EDTA Buffer (REF 700-25)
- **Large scale application:** 0.6×10^9 to 1.2×10^9 CD133 positive cells out of 120×10^9 total cells (WBC): **2 vials** of CliniMACS CD133 Reagent (REF 172-01), CliniMACS Tubing Set LS (REF 162-01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- Label bags as: **Cell Collection, Cell Preparation, Plasma Waste, Wash Waste No. 1** and **Wash Waste No. 2.**
- Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag.

III. Leukapheresis product (LP)

Analysis of LP

Transfer of LP into Cell Preparation Bag

Dilution of LP 1:3 — Add buffer: Amount of buffer to be added = Weight of LP × 2

Centrifugation — 200×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

Volume adjustment — Labeling volume: **95 g (normal scale application)**
190 g (large scale application)

- Remove supernatant to adjust the sample using the following equation:
  Weight of supernatant to be removed = Weight of diluted LP – Labeling volume
- Resuspend the cell pellet.

IV. Magnetic labeling of the cells

Incubation with CliniMACS® CD133 Reagent

Add 1 or 2 vials of CliniMACS® CD133 Reagent (see above), incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent:

- Fill Cell Preparation Bag with buffer.
- Centrifuge (200×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Fill Cell Preparation Bag with buffer.
- Centrifuge (200×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Adjust sample loading volume to: **Normal scale**: 150 g
  Large scale: 275 g

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

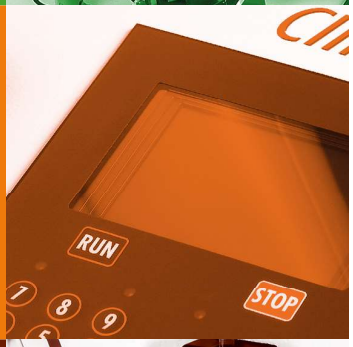
Enrichment of CD133 positive cells

Four STEPS to your target cells

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program CD133 SELECTION 1/2



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Cell Collection Bag

Attach Priming Waste Bag and insert pre-column

Insert separation column and load valve no. 5

Load valves nos. 1, 2, 3, and 4

Load pump tubing

Load valves nos. 7 and 8

Load valves nos. 6, 9, 10, and 11

Recheck all tubing and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment of CD14 positive cells

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD14 Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material

- 4×10^9 CD14 positive cells out of 20×10^9 total cells (WBC):
  1 vial of CliniMACS CD14 Reagent (REF 272-01), CliniMACS Tubing Set TS (REF 161-01), CliniMACS PBS/EDTA Buffer (REF 700-25)
- If the labeling volume (see "Volume adjustment") must be adjusted to 100 g, **2 vials** of CliniMACS CD14 Reagent must be used.

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- Label bags as: **Cell Collection, Cell Preparation, Plasma Waste**, and **Wash Waste.**
- Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag.

III. Leukapheresis product (LP)

Analysis of LP

Transfer of LP into Cell Preparation Bag

Dilution of LP — Add buffer: **Weight of buffer to be added = 600 g – Weight of LP**

Centrifugation — 300×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C)

Volume adjustment — Labeling volume: **50 g or 100 g (if the labeling volume exceeds 50 g)**

- Remove supernatant to adjust the sample using the following equation:
  Weight of supernatant to be removed = Weight of diluted LP – Labeling volume
- Resuspend the cell pellet.

IV. Magnetic labeling of the cells

Incubation with CliniMACS® CD14 Reagent

Add **1** or **2 vial(s)** of CliniMACS® CD14 Reagent (see above), incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent

- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant as much as possible.
- Resuspend the cell pellet.
- Adjust sample loading volume to **100 g.**

37000
ch15/15
(Issued:
2015-08)

Enrichment of CD14 positive cells

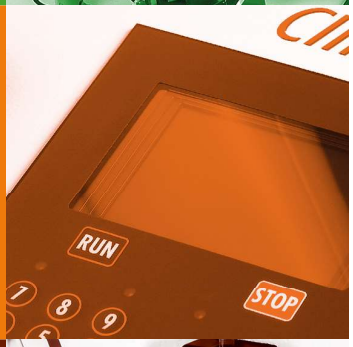
Four STEPS to your target cells

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program ENRICHMENT 1.1

Sample parameter input



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Cell Collection Bag

Attach Priming Waste Bag and insert pre-column

Insert separation column and load valve no. 5

Load valves nos. 1, 2, 3, and 4

Load pump tubing

Load valves nos. 7 and 8

Load valves nos. 6, 9, 10, and 11

Recheck all tubing and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Flexible Labeling with the Anti-Biotin Reagent

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® Anti-Biotin Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material

Enrichment:
- For processing up to 40×10^9 total cells (WBC): 1 vial of CliniMACS Anti-Biotin Reagent (REF 173-01), 1 CliniMACS Tubing Set TS (REF 161-01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Depletion:
- **Normal scale:** For processing 40×10^9 total cells (WBC): **1 vial** of CliniMACS Anti-Biotin Reagent (REF 173-01), 1 CliniMACS Tubing Set LS (REF 162-01), CliniMACS PBS/EDTA Buffer (REF 700-25)
- **Large scale:** For processing 80×10^9 total cells (WBC): **2 vials** of CliniMACS Anti-Biotin Reagent (REF 173-01), 1 CliniMACS Tubing Set LS (REF 162-01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- Label bags as: **Cell Collection**, **Cell Preparation, Plasma Waste**, **Wash Waste No. 1** and **Wash Waste No. 2**.
- Determine the weight of the empty Cell Collection and Cell Preparation Bag.

III. Cellular starting product (CSP)

Analysis of CSP

Transfer of CSP into Cell Preparation Bag

Dilution of CSP — Add buffer: Amount of buffer to be added = Weight of CSP × 2

Centrifugation — 300×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C)
- Remove supernatant to adjust volume for primary antibody labeling as recommended by the manufacturer of the primary antibody.

Volume adjustment
- Adjust the labeling volume to the volume recommended by the manufacturer of the primary antibody.
  Volume to remove = Volume of CSP + Volume of buffer – labeling volume

Primary antibody labeling — Follow the instructions for use provided by the manufacturer of the primary antibody.

1st wash
- Add buffer: Weight of buffer to be added = 600 g – Weight of CSP
- Centrifuge (300×g, without brake, 15 min, RT).

Volume Adjustment — Labeling volume: 95 g (normal scale application)
190 g (large scale application)
- Remove supernatant to adjust the sample using the following equation:
  Weight of supernatant to be removed = Weight of diluted CSP – Labeling volume
- Resuspend the cell pellet.

IV. Magnetic labeling of the cells

Incubation with CliniMACS® Anti-Biotin Reagent

Add 1 or 2 vials of CliniMACS® Anti-Biotin Reagent, incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent: 2nd wash
- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Adjust volume: **100 g** (Normale scale application) or
  200 g (Large scale application).

Four STEPS to your target cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Flexible Labeling with the Anti-Biotin Reagent

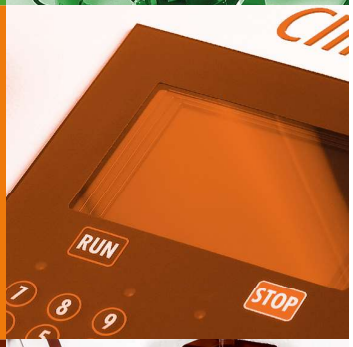
Four STEPS to your target cells

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program ENRICHMENT 1.1 or DEPLETION 2.1

Sample parameter input



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Cell Collection Bag

Attach Priming Waste Bag and insert pre-column

Insert separation column and load valve no. 5

Load valves nos. 1, 2, 3, and 4

Load pump tubing

Load valves nos. 7 and 8

Load valves nos. 6, 9, 10, and 11

Recheck all tubing and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment of CD56 positive cells

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD56 Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material — Up to 5×10^9 positive cells out of 40×10^9 total cells (WBC):
1 vial of CliniMACS CD56 Reagent (REF 172-01), 1 CliniMACS Tubing Set TS (REF 161-01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- Label bags as: **Cell Collection**, **Cell Preparation, Plasma Waste**, and **Wash Waste**.
- Determine the weight of the empty Cell Collection and Cell Preparation Bag.

III. Leukapheresis product (LP)

Analysis of LP

Transfer of LP into Cell Preparation Bag

Dilution of LP — Add buffer: Weight of buffer to be added = 600 g – Weight of LP

Centrifugation — 200×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

If the cell source has been previously processed (e.g. CD3 depletion) centrifuge the cells at 300×g for 15 min without brake, at room temperature.

Volume adjustment — Labeling volume: **95 g**

- Remove the supernatant completly taking care not to resuspend the cell pellet.
- Resuspend the cell pellet carefully.
- Adjust the volume to 95 g by adding buffer to the Cell Preparation Bag (CPB) using the following equation:
  Target weight of filled CPB = 95 g + Weight of empty CPB
- Resuspend cell pellet.

IV. Magnetic labeling of the cells

Incubation with CliniMACS® CD56 Reagent

Add 1 vial of CliniMACS® CD56 Reagent, incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent:

- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Adjust sample loading volume: **150 g**

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment of CD56 positive cells

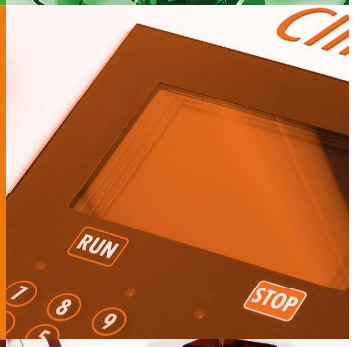
Four STEPS to your target cells

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program ENRICHMENT 1.1

Sample parameter input



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Cell Collection Bag

Attach Priming Waste Bag and insert pre-column

Insert separation column and load valve no. 5

Load valves nos. 1, 2, 3, and 4

Load pump tubing

Load valves nos. 7 and 8

Load valves nos. 6, 9, 10, and 11

Recheck all tubing and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

Depletion of CD3 positive cells with the CliniMACS® Tubing Set LS

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD3 Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material

Normal scale preparation: Up to 15×10^9 positive cells out of 40×10^9 total cells (WBC):
1 vial of CliniMACS CD3 Reagent (REF 273-01), 1 CliniMACS Tubing Set LS (REF 162-01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Large scale preparation: Up to 20×10^9 positive cells out of 80×10^9 total cells (WBC):
2 vials of CliniMACS CD3 Reagent (REF 273-01), 1 CliniMACS Tubing Set LS (REF 162-01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- Label bags as: **Cell Collection**, **Cell Preparation, Plasma Waste**, and **Wash Waste**.
- Determine the weight of the empty Cell Collection and Cell Preparation Bag.

III. Leukapheresis product (LP)

Analysis of LP

Determine the number of all CD3 labeled cells (including unspecific CD3 binding, not only real T cells).
Consider this number for application specifications and sample parameter input (see STEP 2).

Transfer of LP into Cell Preparation Bag (CPB)

Dilution of LP

Add buffer: Weight of buffer to be added = 600 g – Weight of LP

Centrifugation

200×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

Volume adjustment

Labeling volume: **95 g (Normal scale application)**
190 g (Large scale application)

- Remove supernatant completely taking care not to resuspend the cell pellet.
- Resuspend the cell pellet carefully.
- Adjust volume by adding buffer to the Cell Preparation Bag (CPB) using the following equation (consider IgG volume, see IV.):
  Normal scale: Target weight of filled CPB = 95 g – IgG weight + Weight of empty CPB
  Large scale: Target weight of filled CPB = 190 g – IgG weight + Weight of empty CPB
- Resuspend cell pellet.

IV. Magnetic labeling of the cells

Incubation with clinical grade immunoglobulin

Add IgG (1.5 mg/mL), incubate on orbital rotator (25 rpm) for 5 min at RT.

Incubation with CliniMACS® CD3 Reagent

Add 1 or 2 vials of CliniMACS CD3 Reagent (see above), incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent:

- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Adjust sample loading volume to **150 g (Normale scale application)** or **250–300 g (Large scale application).**

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Four STEPS to your target cells

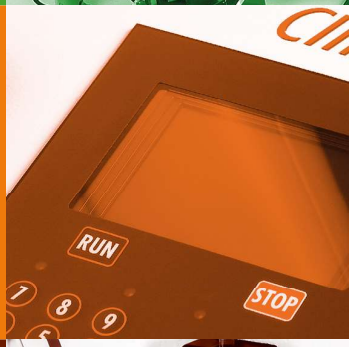
Depletion of CD3 positive cells with the CliniMACS® Tubing Set LS

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program DEPLETION 2.1

Sample parameter input



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Cell Collection Bag

Attach Priming Waste Bag and insert pre-column

Insert separation column and load valve no. 5

Load valves nos. 1, 2, 3, and 4

Load pump tubing

Load valves nos. 7 and 8

Load valves nos. 6, 9, 10, and 11

Recheck all tubing and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Depletion of CD3 positive cells with the CliniMACS® Depletion Tubing Set

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD3 Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material

Normal scale preparation: Up to 15×10^9 positive cells out of 40×10^9 total cells (WBC):
1 vial of CliniMACS CD3 Reagent (REF 273-01), 1 CliniMACS Depletion Tubing Set (REF 261-01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Large scale preparation: Up to 30×10^9 positive cells out of 80×10^9 total cells (WBC):
2 vials of CliniMACS CD3 Reagent (REF 273-01), 1 CliniMACS Depletion Tubing Set (REF 261-01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- Label bags as: **Cell Preparation, Plasma Waste**, and **Wash Waste**.
- Determine the weight of the empty Cell Preparation Bag.

III. Leukapheresis product (LP)

Analysis of LP

Determine the number of all CD3 labeled cells (including unspecific CD3 binding, not only real T cells).
Consider this number for application specifications and sample parameter input (see STEP 2).

Transfer of LP into Cell Preparation Bag (CPB)

Dilution of LP

Add buffer: Weight of buffer to be added = 600 g – Weight of LP

Centrifugation

200×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

Volume adjustment

Labeling volume: **95 g (Normal scale application)**
190 g (Large scale application)

- Remove supernatant completely taking care not to resuspend the cell pellet.
- Resuspend the cell pellet carefully.
- Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation (consider IgG weight, see IV.):
  Target weight of filled CPB = Labeling volume – IgG weight + Weight of empty CPB
- Resuspend cell pellet.

IV. Magnetic labeling of the cells

Incubation with clinical grade immunoglobulin

Add IgG (1.5 mg/mL), incubate on orbital rotator (25 rpm) for 5 min at RT.

Incubation with CliniMACS® CD3 Reagent

Add **1 or 2 vials** of CliniMACS CD3 Reagent (see above), incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent:

- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Adjust sample loading volume to **150 g** (Normale scale application) or **250–300 g** (Large scale application).

37000
ch15/15
(Issued:
2015-08)

Four STEPS to your target cells

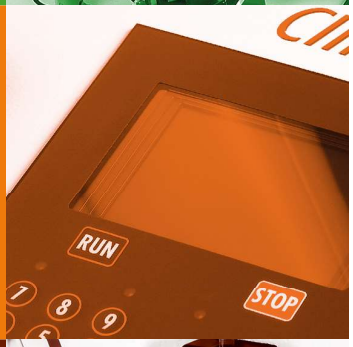
Depletion of CD3 positive cells with the CliniMACS® Depletion Tubing Set

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program DEPLETION 3.1

Sample parameter input
(according to rough analysis (see STEP 1, III. Leukapheresis product))



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Non-Target Cell Bag, Reapplication Bag, and insert separation column

Load valves nos. 1, 2, 3, 4, and 5

Load pump tubing

Load valves nos. 6, 7, 8, 9, and 10

Recheck all tubing and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

Depletion of CD19 positive cells

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD19 Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material

Normal scale preparation: Up to 5×10^9 positive cells out of 40×10^9 total cells (WBC):
1 vial of CliniMACS CD19 Reagent (REF 179-01), 1 CliniMACS Tubing Set LS (REF 162-01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Large scale preparation: Up to 10×10^9 positive cells out of 80×10^9 total cells (WBC):
2 vials of CliniMACS CD19 Reagent (REF 179-01), 1 CliniMACS Tubing Set LS (REF 162-01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- Label bags as: **Cell Collection**, **Cell Preparation, Plasma Waste**, and **Wash Waste**.
- Determine the weight of the empty Cell Collection and Cell Preparation Bag.

III. Leukapheresis product (LP)

Analysis of LP

Transfer of LP into Cell Preparation Bag (CPB)

Dilution of LP — Add buffer: Weight of buffer to be added = 600 g – Weight of LP

Centrifugation — 200×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

Volume adjustment — Labeling volume: **95 g (Normal scale application)**
190 g (Large scale application)

- Remove supernatant completely taking care not to resuspend the cell pellet.
- Resuspend the cell pellet carefully.
- Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation:
  Normal scale: Target weight of filled CPB = 95 g + Weight of empty CPB
  Large scale: Target weight of filled CPB = 190 g + Weight of empty CPB
- Resuspend cell pellet.

IV. Magnetic labeling of the cells

Incubation with CliniMACS® CD19 Reagent

Add 1 or 2 vials of CliniMACS® CD19 Reagent (see above), incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent:

- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Adjust sample loading volume to **100 g (Normale scale application)** or **200 g (Large scale application)**.

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Four STEPS to your target cells

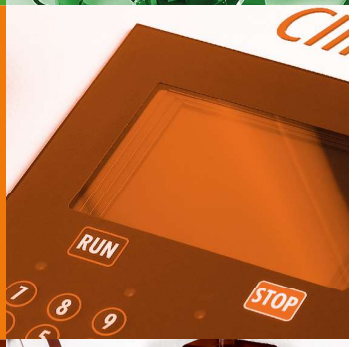
Depletion of CD19 positive cells

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program DEPLETION 2.1

Sample parameter input



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Cell Collection Bag

Attach Priming Waste Bag and insert pre-column

Insert separation column and load valve no. 5

Load valves nos. 1, 2, 3, and 4

Load pump tubing

Load valves nos. 7 and 8

Load valves nos. 6, 9, 10, and 11

Recheck all tubing and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Depletion of CD3 positive cells and CD19 positive cells with the CliniMACS® Tubing Set LS

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD3 Reagent, CliniMACS® CD19 Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material Up to 15×10^9 CD3 positive cells and up to 5×10^9 CD19 positive cells out of 40×10^9 total cells:
1 vial of CliniMACS CD3 Reagent (REF 273-01), **1 vial** of CliniMACS CD19 Reagent (REF 179-01), CliniMACS Tubing Set LS (REF 162-01), CliniMACS PBS/EDTA Buffer (REF 700-25).

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- Label bags as: **Cell Collection**, **Cell Preparation, Plasma Waste**, and **Wash Waste**.
- Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag.

III. Leukapheresis product (LP)

Analysis of LP Determine the number of all CD3 and CD19 labeled cells (including unspecific CD3 and CD19 binding, not only real T and B cells).
Consider this number for application specifications and sample parameter input (see STEP 2).

Transfer of LP into Cell Preparation Bag (CPB)

Dilution of LP Add buffer: Weight of buffer to be added = 600 g – Weight of LP

Centrifugation 200×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

Volume adjustment Labeling volume 87.5 g

- Remove supernatant completely taking care not to resuspend the cell pellet.
- Resuspend the cell pellet carefully.
- Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation (consider IgG volume, see IV.):
  Target weight of filled CPB = 87.5 g – IgG weight + Weight of empty CPB
- Resuspend cell pellet.

IV. Magnetic labeling of the cells

Incubation with clinical grade immunoglobulin

Add IgG (1.5 mg/mL), incubate on orbital rotator (25 rpm) for 5 min at RT.

Incubation with CliniMACS® CD3 Reagent and the CliniMACS® CD19 Reagent

Add 1 vial of CliniMACS CD3 Reagent and 1 vial CliniMACS CD19 Reagent, incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent:

- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Adjust sample loading volume to: **150 g**

37000
ch15/15
(Issued:
2015-08)

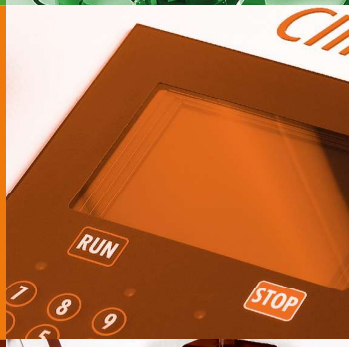
CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Four STEPS to your target cells

Depletion of CD3 positive cells and CD19 positive cells with the CliniMACS® Tubing Set LS

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument
Choice of separation program DEPLETION 2.1
Sample parameter input
(according to rough analysis (see STEP 1, III. leukaphesis product))



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation
Attach Cell Collection Bag
Attach Priming Waste Bag and insert pre-column
Insert separation column and load valve no. 5
Load valves nos. 1, 2, 3, and 4
Load pump tubing
Load valves nos. 7 and 8
Load valves nos. 6, 9, 10, and 11
Recheck all tubing and attachments
Seating of valves
Attach CliniMACS® PBS/EDTA Buffer
Start priming
Check during the priming
Final check of all tubing and attachments
Integrity test
Connect Cell Preparation Bag
Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure
Disconnect bags and record process code
Unload tubing set and shutdown
Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Depletion of CD3 positive cells and CD19 positive cells with the CliniMACS® Depletion Tubing Set

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD3 Reagent, CliniMACS® CD19 Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material

Normal scale preparation: Up to 15×10^9 CD3 positive cells and up to 5×10^9 CD19 positive cells out of 40×10^9 total cells: **1 vial** of CliniMACS CD3 Reagent (REF 273-01), **1 vial** of CliniMACS CD19 Reagent (REF 179-01), CliniMACS Depletion Tubing Set (REF 261-01), CliniMACS PBS/EDTA Buffer (REF 700-25).

Large scale preparation: Up to 30×10^9 CD3 positive cells and up to 10×10^9 CD19 positive cells out of 80×10^9 total cells: **2 vials** of CliniMACS CD3 Reagent (REF 273-01), **2 vials** of CliniMACS CD19 Reagent (REF 179-01), CliniMACS Depletion Tubing Set (REF 261-01), CliniMACS PBS/EDTA Buffer (REF 700-25).

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- Label bags as: **Cell Preparation, Plasma Waste**, and **Wash Waste**.
- Determine the weight of the empty Cell Collection and Cell Preparation Bag.

III. Leukapheresis product (LP)

Analysis of LP

Determine the number of all CD3 and CD19 labeled cells (including unspecific CD3 and CD19 binding, not only real T and B cells).
Consider this number for application specifications and sample parameter input (see STEP 2).

Transfer of LP into Cell Preparation Bag (CPB)

Dilution of LP

Add buffer: Weight of buffer to be added = 600 g – Weight of LP

Centrifugation

200×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

Volume adjustment

Labeling volume: **87.5 g (Normal scale application)**
175 g (Large scale application)

- Remove supernatant completely taking care not to resuspend the cell pellet.
- Resuspend the cell pellet carefully.
- Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation (consider IgG volume, see IV.):
  Normal scale: CPB Target weight = 87.5 g – IgG weight + Weight of empty CPB
  Large scale: CPB Target weight = 175 g – IgG weight + Weight of empty CPB
- Resuspend cell pellet.

IV. Magnetic labeling of the cells

Incubation with clinical grade immunoglobulin

Add IgG (1.5 mg/mL), incubate on orbital rotator (25 rpm) for 5 min at RT.

Incubation with CliniMACS® CD3 and CliniMACS® CD19 Reagent

Add **1 or 2 vials** of CliniMACS CD3 Reagent and **1 or 2 vials** of CliniMACS CD19 Reagent (see above), incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent:

- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Adjust sample loading volume to **150 g (Normale scale application)** or **250–300 g (Large scale application).**

37000
ch15/15
(Issued:
2015-08)

Four STEPS to your target cells

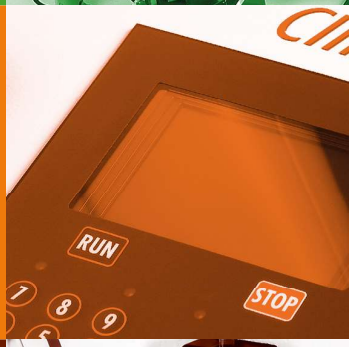
Depletion of CD3 positive cells and CD19 positive cells with the CliniMACS® Depletion Tubing Set

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program DEPLETION 3.1

Sample parameter input
(according to rough analysis (see STEP 1, III. Leukapheresis product))



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Non-Target Cell Bag, Reapplication Bag, and insert separation column

Load valves nos. 1, 2, 3, 4, and 5

Load pump tubing

Load valves nos. 6, 7, 8, 9, and 10

Recheck all tubings and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment/Depletion of CD8 positive cells

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD8 Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material

Enrichment:

- **Normal scale application:** Up to 4×10^9 labeled cells out of 40×10^9 total cells (WBC): 1 vial of CliniMACS CD8 Reagent (REF 275-01), 1 CliniMACS Tubing Set TS (REF 161- 01), CliniMACS PBS/EDTA Buffer (REF 700-25)
- **Large scale application:** 4 to 5×10^9 labeled cells out of 80×10^9 total cells (WBC): 2 vials of CliniMACS CD8 Reagent (REF 275-01), 1 CliniMACS Tubing Set LS (REF 162- 01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Depletion:

- **Normal scale application:** Up to 4×10^9 labeled cells out of 40×10^9 total cells (WBC): 1 vial of CliniMACS CD8 Reagent (REF 275-01), 1 CliniMACS Tubing Set LS (REF 162- 01), CliniMACS PBS/EDTA Buffer (REF 700-25)
- **Large scale application:** 4 to 8×10^9 labeled cells out of 80×10^9 total cells (WBC): 2 vials of CliniMACS CD8 Reagent (REF 275-01), 1 CliniMACS Tubing Set LS (REF 162- 01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- Label bags as: **Cell Collection**, **Cell Preparation, Plasma Waste**, and **Wash Waste**.
- Determine the weight of the empty Cell Collection and Cell Preparation Bag.

III. Leukapheresis product (LP)

Analysis of LP

Transfer of LP into Cell Preparation Bag (CPB)

Dilution of LP — Add buffer: **Weight of buffer to be added = 600 g – Weight of LP**

Centrifugation — 300×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

Volume adjustment — Labeling volume: **95 g (Normal scale application)**
190 g (Large scale application)

- Remove supernatant completely taking care not to resuspend the cell pellet.
- Resuspend the cell pellet carefully.
- Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation:
  Target weight of filled CPB = Labeling volume + Weight of empty CPB
- Resuspend cell pellet.

IV. Magnetic labeling of the cells

Incubation with CliniMACS® CD8 Reagent

Add 1 or 2 vials of CliniMACS CD8 Reagent (see above), incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent:

- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Adjust sample loading volume to **100 g (Normale scale application)** or **200 g (Large scale application)**.

- Note: If the number of calculated cells exceeds the separation capacity of the tubing set, split the sample and continue with each sample separately, using a new tubing set for every CliniMACS run.

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment/Depletion of CD8 positive cells

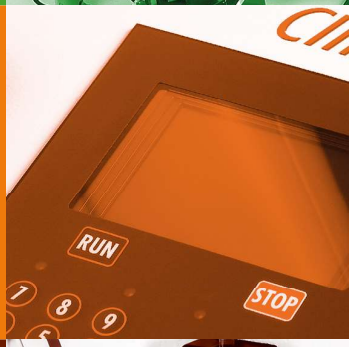
Four STEPS to your target cells

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program ENRICHMENT 1.1 or DEPLETION 2.1

Sample parameter input



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Cell Collection Bag

Attach Priming Waste Bag and insert pre-column

Insert separation column and load valve no. 5

Load valves nos. 1, 2, 3, and 4

Load pump tubing

Load valves nos. 7 and 8

Load valves nos. 6, 9, 10, and 11

Recheck all tubing and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment of IFN-gamma secreting T cells with the CliniMACS® Cytokine Capture System (IFN-gamma)

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® Cytokine Capture System (IFN-gamma), Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material IFN-gamma secreting cells from out of 1×10^9 total cells (WBC): 1 box of CliniMACS® Cytokine Capture System (IFN-gamma) (REF 279-01), CliniMACS Tubing Set TS (REF 161-01), CliniMACS® PBS/EDTA Buffer (REF 700-25)

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- ☐ Label bags as: **Cell Collection**, **Cell Preparation No. 1 (Start), Cell Preparation No. 2 (Secretion)**, **Plasma Waste No. 1,** and **Plasma Waste No. 2.** Determine the weight of the empty Cell Collection and Cell Preparation Bags.
- ☐ Label wash waste bags as: **Wash Waste No. 1, Wash Waste No. 2, Wash Waste No. 3, Wash Waste No. 4, Wash Waste No. 5, Wash Waste No. 6,** and **Wash Waste No. 7.**
- ☐ Prepare two 600 mL transfer bags, each with 500 mL of cold medium (+2 °C to +8 °C). Label bags as: **Medium**.
- ☐ Prepare one 600 mL transfer bag with 600 mL of medium (RPMI), supplemented with 10% AB serum. Pre-warm at +37 °C. Label bag as: **Medium 10%**.
- ☐ Prepare one 600 mL transfer bag with 500 mL of medium (RPMI), supplemented with 2% AB serum, at +4 °C. Label bag as: **Medium 2%**.
- ☐ Prepare four 600 mL transfer bags, each prefilled with 125 mL of culture medium, supplemented with 10% AB serum. Pre-warm at +37 °C. Label one of those bags as: **Cell Preparation No. 3 (Separation)**.

III. Cellular starting product (CSP)

Transfer of CSP into Cell Preparation Bag No. 1

Dilution of CSP
Add cold (+2 to +8 °C) medium up to 500 mL:
Weight of medium to be added (g) = 500 g – Weight of CSP (g)

Centrifugation
200×g, 10 min, without brake, room temperature (RT: +19 °C to +25 °C).
- ☐ Remove supernatant completely taking care not to resuspend the cell pellet.
- ☐ Repeat dilution and centrifugation.

Volume adjustment
Labeling volume: 100 mL
- ☐ Adjust the volume to 100 g using the following equation:
  Target volume = 95 g + Weight of empty Cell Preparation Bag
- ☐ Add pre-warmed (+37 °C) culture medium suppl. with 10% AB serum.
- ☐ Transfer cells into gas-permeable cell culture bag.

IV. Labeling of the cells

In vitro restimulation

If *in vitro* re-stimulation is necessary, this is always performed according to validated procedures established by the user of the CliniMACS Cytokine Capture System (IFN-gamma). Generally, re-stimulation is performed using appropriate cell culture medium with a usual volume of the cell sample of 100 ml, and a cell dencity of 0.5-1x10^7 cells/cm2 and 1x10^7 cells/mL.

Labeling with the CliniMACS® IFN-gamma Catchmatrix Reagent

Transfer cells into Cell Preparation Bag No. 2. Fill the bag up to 600 mL with cold (+2 to +8 °C) culture medium suppl. with 2% AB serum. Centrifuge (300×g, 10 min, +4 to +8 °C, without brake). Carefully remove supernatant completely. Resuspend cell pellet in the residual volume after removal. Add CliniMACS® IFN-gamma Catchmatrix Reagent by injection, incubate on ice (5 min).

Secretion phase

- ☐ Fill Cell Preparation Bag No. 2 with 500 mL of pre-warmed (+37 °C) culture medium suppl. with10% AB serum.
- ☐ Start masuring incubation time.
- ☐ Distribute 125 mL suspension to the 4 bags pre-filled with 125 mL of culture medium suppl. with 10 % AB serum.
- ☐ Incubate on orbital shaker (max. 50 rpm, 45 min, +37 °C) until the end of incubation time.
- ☐ After the 45 min, add cold (+2 to +8 °C) buffer suppl. with HSA (at least 1 volume, i.e. 250 mL each).
- ☐ Place on ice for 10 min.
- ☐ Centrifuge (300×g, 10 min, +4 °C, without brake). Carefully remove supernatant completely.
- ☐ Recombine cell suspension in the Cell Preparation Bag No. 3 and fill the bag up to 500 mL with cold (+2 °C to +8 °C) buffer.
- ☐ Centrifuge (300×g, 10 min, +4 °C, without brake). Carefully remove supernatant completely.

Magnetic labeling of cells with CliniMACS® IFN-gamma Enrichment Reagent

After removal of the supernatant resuspend pellet in residual volume. Add CliniMACS® IFN-gamma Enrichment Reagent by injection. Immediately start measuring incubation time (15 min). Incubate for 15 min on ice. Fill bag up to 500 mL of cold (+2 to +8 °C) buffer. Centrifuge (300×g, +4 °C, 10 min, no brake) and carefully remove supernatant completely. Resuspend the cells in 100 mL of cold (+2 to +8 °C) buffer.

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

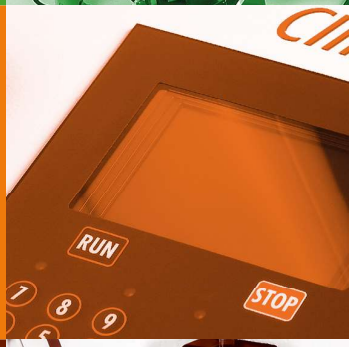
Four STEPS to your target cells

Enrichment of IFN-gamma secreting T cells with the CliniMACS® Cytokine Capture System (IFN-gamma)

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of ENRICHMENT 3.2



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Cell Collection Bag

Attach Priming Waste Bag and insert pre-column

Insert separation column and load valve no. 5

Load valves nos. 1, 2, 3, and 4

Load pump tubing

Load valves nos. 7 and 8

Load valves nos. 6, 9, 10, and 11

Recheck all tubing and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment/Depletion of CD25 positive cells

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD25 Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material

Enrichment:

- **CD25 positive or CD25 highly expressing cells:** Up to 0.6×10^9 labeled cells out of 40×10^9 total cells (WBC): **1 vial** CliniMACS® CD25 Reagent (REF 274-01), CliniMACS® Tubing Set (REF 161-01), CliniMACS® PBS/EDTA Buffer (REF 700-25)

Depletion:

- **Normal scale:** Up to 6×10^9 CD25 positive cells out of 40×10^9 total cells (WBC): **1 vial** of CliniMACS CD25 Reagent (REF 274-01), 1 CliniMACS Tubing Set LS (REF 162- 01), CliniMACS PBS/EDTA Buffer (REF 700-25)
- **Large scale:** 6 to 12×10^9 labeled cells out of 80×10^9 total cells (WBC): **2 vials** of CliniMACS CD25 Reagent (REF 274-01), 1 CliniMACS Tubing Set LS (REF 162- 01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).
Keep the buffer for the cell preparation at room temperature (+19 °C to +25 °C). The magnetic labeling for the enrichment of CD25 highly expressing cells has to be performed using cold buffer (+4°C to +8°C).

Labeling and preparation of bags

- Label bags as: **Cell Collection**, **Cell Preparation, Plasma Waste**, and **Wash Waste**.
- Determine the weight of the empty Cell Collection and Cell Preparation Bag.

III. Leukapheresis product (LP)

Analysis of LP

Transfer of LP into Cell Preparation Bag (CPB)

Dilution of LP 1:3 — Add buffer: Weight of buffer to be added = Weight of LP × 2

Centrifugation — 300×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

Volume adjustment — Labeling volume for **enrichment of CD25 positive cells: 190 g**
enrichment of CD25 highly expressing cells: 380 g *
depletion of CD25 positive cells (normale scale): 95 g
depletion of CD25 positive cells (large scale): 190 g

* The magnetic labeling for the enrichment of CD25 highly expressing cells has to be performed using cold buffer (+4°C to +8°C).

- Remove supernatant completely taking care not to resuspend the cell pellet.
- Resuspend the cell pellet carefully.
- Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation:
  Target weight of filled CPB = labeling volume + Weight of empty CPB
- Resuspend cell pellet.

IV. Magnetic labeling of the cells

Incubation with CliniMACS® CD25 Reagent

Add 1 or 2 vials of CliniMACS CD25 Reagent, incubate on orbital rotator (25 rpm).

a) **Enrichment or depletion of CD25 positive cells**: 30 minutes at RT.
b) **Enrichment of CD25 highly expressing cells**: 15 minutes at +4 °C to +8 °C.

Removal of excess reagent:

- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Adjust sample loading volume to 100 g (Normale scale application using one vial) or 200 g (Large scale application using two vials).

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment/Depletion of CD25 positive cells

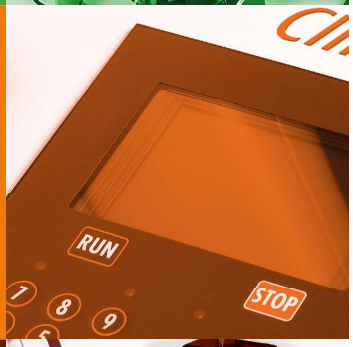
Four STEPS to your target cells

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program ENRICHMENT 3.2 or DEPLETION 2.1

Sample parameter input (not for ENRICHMENT 3.2)



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Cell Collection Bag

Attach Priming Waste Bag and insert pre-column

Insert separation column and load valve no. 5

Load valves nos. 1, 2, 3, and 4

Load pump tubing

Load valves nos. 7 and 8

Load valves nos. 6, 9, 10, and 11

Recheck all tubing and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment/Depletion of CD4 positive cells

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD4 Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material

Enrichment:
- Up to 5×10^9 labeled cells out of 40×10^9 total cells (WBC):
  1 vial of CliniMACS CD4 Reagent (REF 276-01), 1 CliniMACS Tubing Set TS (REF 161- 01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Depletion:
- **Normal scale application:** Up to 12×10^9 labeled cells out of 40×10^9 total cells (WBC):
  1 vial of CliniMACS CD4 Reagent (REF 276-01), 1 CliniMACS Tubing Set LS (REF 162- 01), CliniMACS PBS/EDTA Buffer (REF 700-25)
- **Large scale application:** 12 to 20×10^9 labeled cells out of 80×10^9 total cells (WBC):
  2 vials of CliniMACS CD4 Reagent (REF 276-01), 1 CliniMACS Tubing Set LS (REF 162- 01), CliniMACS PBS/EDTA Buffer (REF 700-25)
- **Note:** If the number of calculated cells exceeds the application capacity (see above), split the sample and continue with each sample separately, using a new tubing set for every CliniMACS Run.

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- Label bags as: **Cell Collection**, **Cell Preparation, Plasma Waste**, and **Wash Waste**.
- Determine the weight of the empty Cell Collection and Cell Preparation Bag.

III. Leukapheresis product (LP)

Analysis of LP

Transfer of LP into Cell Preparation Bag

Dilution of LP 1:3 — Add buffer: Weight of buffer to be added = Weight of LP × 2

Centrifugation — 300×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

Volume adjustment — Labeling volume: **95 g (Normal scale application)**
190 g (Large scale application)

- Remove supernatant to adjust the sample using the following equation:
  Weight of supernatant to be removed = Weight of diluted LP – Labeling volume
- Remove supernatant taking care not to resuspend the cell pellet.
- Resuspend cell pellet.

IV. Magnetic labeling of the cells

Incubation with CliniMACS® CD4 Reagent

Add 1 or 2 vials of CliniMACS CD4 Reagent (see above), incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent:

- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend the cell pellet.
- Adjust sample loading volume to **100 g (Normale scale application)** or **200 g (Large scale application)**.

37000
ch15/15
(Issued:
2015-08)

Enrichment/Depletion of CD4 positive cells

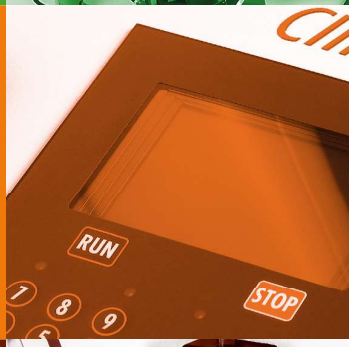
Four STEPS to your target cells

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program ENRICHMENT 1.1 or DEPLETION 2.1

Sample parameter input



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Cell Collection Bag

Attach Priming Waste Bag and insert pre-column

Insert separation column and load valve no. 5

Load valves nos. 1, 2, 3, and 4

Load pump tubing

Load valves nos. 7 and 8

Load valves nos. 6, 9, 10, and 11

Recheck all tubing and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment of CD133 positive cells (Bone Marrow)

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD133 Reagent (Bone Marrow), Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material

For 50 mL bone marrow aspirate:

- **1 vial** CliniMACS CD133 Reagent (REF 172-01), CliniMACS Tubing Set TS (REF 161-01), CliniMACS PBS/EDTA Buffer (REF 700-25)
- If the labeling volume (see "Volume adjustment") must be adjusted to 190 mL, **2 vials** of CliniMACS CD133 Reagent must be used.

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- Label bags as: **Cell Collection**, **Bone Marrow Aspirate**, **Cell Preparation, Plasma Waste**, **Wash Waste No. 1** and **Wash Waste No. 2**.
- Determine the weight of the empty Cell Collection and Cell Preparation Bag.

III. Bone Marrow Aspirate (BMA)

Analysis of BMA

Filtration of BMA and transfer into Cell Preparation Bag

Dilution of BMA 1:5 — Add buffer: **Weight of buffer to be added = Weight of BMA × 4**

Centrifugation — 300×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

Volume adjustment — Labeling Volume: **95 g** (or 190 g)

- Remove supernatant to adjust the sample using the following equation:
  Weight of supernatant to be removed = Weight of BMA – 95 g
- If the recommended labeling volume exceeds 95 g, adjust the sample to 190 g by adding buffer using the following equation:
  Weight of buffer to be added = 190 g – Weight of diluted BMA
- Resuspend the cell pellet.

IV. Magnetic labeling of the cells

Incubation with clinical grade immunoglobulin

Add IgG (1.5 mg/mL), incubate on orbital rotator (25 rpm) for 5 min at RT. After adding the human IgG solution, the total volume should not exceeded 100 mL for a 95 mL cellular product, or 195 mL for a 190 mL cellular product.

Incubation with CliniMACS® CD133 Reagent

Add **1 vial** of CliniMACS CD133 Reagent if the labeling volume was adjusted to 95 mL or **2 vials** if the labeling volume is 190 mL , incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent:

- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, 15 min, RT, moderate brake).
- Remove supernatant.
- Resuspend cell pellet.
- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, 15 min, RT, moderate brake).
- Remove supernatant.
- Resuspend cell pellet.
- Adjust sample loading volume to **100 g**.
  (Adjust the volume if the weight of the BMA is less than 100 g. The volume may range between 100 g and 275 g but must not exceed 275 g).

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

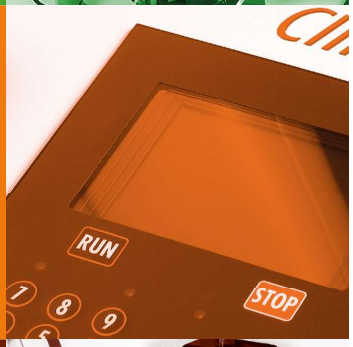
Enrichment of CD133 positive cells (Bone Marrow)

Four STEPS to your target cells

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program CD133 Selection 1/2



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Cell Collection Bag

Attach Priming Waste Bag and insert pre-column

Insert separation column and load valve no. 5

Load valves nos. 1, 2, 3, and 4

Load pump tubing

Load valves nos. 7 and 8

Load valves nos. 6, 9, 10, and 11

Recheck all tubing and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Depletion of TCRα/β positive cells

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® TCRα/β-Biotin, CliniMACS® Anti-Biotin Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material
For up to 24×10^9 TCRα/β positive cells out of 60×10^9 total cells (WBC):
One CliniMACS TCRα/β Kit (REF 200-070-407) (containing: **1 vial** of CliniMACS® TCRα/β-Biotin (REF 701-48) and **2 vials** of CliniMACS® Anti-Biotin Reagent (REF 173-01)),
CliniMACS® Depletion Tubing Set (REF 261-01), CliniMACS® PBS/EDTA Buffer (REF 700-25)

Preparation of the CliniMACS PBS/EDTA Buffer
Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags
- Label bags as: **Cell Preparation, Plasma Waste**, and **Wash Waste No. 1, No. 2** and **No 3**.
- Determine the weight of the empty Cell Preparation Bag.

III. Leukapheresis product (LP)

Analysis of LP

Transfer of LP into Cell Preparation Bag

Dilution of LP 1:3
Add buffer: **Weight of buffer to be added = Weight of LP × 2**

Centrifugation
200×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

Volume adjustment
Labeling volume **95 g**
- Remove supernatant completely taking care not to resuspend the cell pellet.
- Resuspend the cell pellet carefully.
- Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation: **Target weight of filled CPB = 95 g + Weight of empty CPB**
- Resuspend cell pellet.

IV. Labeling of the cells with the CliniMACS® TCRα/β-Biotin

Incubation with CliniMACS® TCRα/β-Biotin
Add 1 vial of CliniMACS® TCRα/β-Biotin, incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess conjugate: **Repeat this step**.
- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant as much as possible.
- Resuspend cell pellet and adjust volume to **190 g** after second wash.

V. Magnetic labeling of the cells with the CliniMACS® Anti-Biotin Reagent

Incubation with CliniMACS® Anti-Biotin Reagent
Add 2 vials of CliniMACS® Anti-Biotin Reagent, incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent:
- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant as much as possible.
- Resuspend cell pellet.
- Adjust sample loading volume to **150 g** .
  (A maximum WBC concentration of 0.4×10^9 WBC/mL is recommended.)

37000
ch15/15
(Issued:
2015-08)

Four STEPS to your target cells

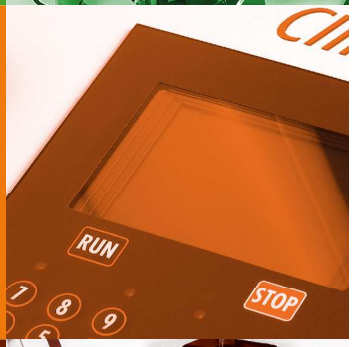
Depletion of TCRα/β positive cells

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program DEPLETION 3.1

Sample parameter input



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Non-Target Cell Bag, Reapplication Bag, and insert separation column

Load valves nos. 1, 2, 3, 4, and 5

Load pump tubing

Load valves nos. 6, 7, 8, 9, and 10

Recheck all tubings and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Depletion of CD45RA positive cells

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD45RA Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material

Up to 20×10^9 positive cells out of 50×10^9 total cells (WBC):
1 vial of CliniMACS CD45RA Reagent (REF 701-46), 1 CliniMACS Depletion Tubing Set (REF 261-01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- Label bags as: **Cell Preparation, Plasma Waste**, and **Wash Waste**.
- Determine the weight of the empty Cell Preparation Bag.

III. Leukapheresis product (LP)

Analysis of LP

Transfer of LP into Cell Preparation Bag

Dilution of LP

Add buffer: **Weight of buffer to be added = Weight of LP × 2**

Centrifugation

200×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

If the negative fraction of a CliniMACS® CD34 Separation is used as alternative starting cell source the dilution step can be skipped. Please continue with the centrifugation step and centrifuge the cells at 300xg for 15 min (without brake) at room temperature (+19 °C to +25 °C).

Volume adjustment

Labeling volume: **95 g**

- Remove supernatant completely taking care not to resuspend the cell pellet.
- Resuspend the cell pellet carefully.
- Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation:
  Target weight of filled CPB = 95 g + Weight of empty CPB
- Resuspend cell pellet.

IV. Magnetic labeling of the cells

Incubation with CliniMACS® CD45RA Reagent

Add **1 vial** of CliniMACS® CD45RA Reagent, incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent:

- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Adjust sample loading volume to **125 g**.
  (Cell concentration should not exceed 0.4×10^9 WBC/mL.)

37000
ch15/15
(Issued:
2015-08)

Depletion of CD45RA positive cells

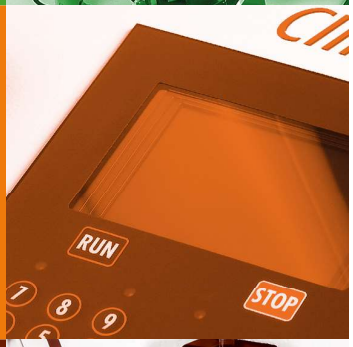
Four STEPS to your target cells

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program DEPLETION 3.1

Sample parameter input



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Non-Target Cell Bag, Reapplication Bag and insert separation column

Load valves nos. 1, 2, 3, 4 and 5

Load pump tubing

Load valves nos. 6, 7, 8, 9 and 10

Recheck all tubings and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment of CD271 positive cells (Bone Marrow)

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD271 Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material

100 mL bone marrow aspirate:

- 1 vial CliniMACS CD271 Reagent (REF 701-12), CliniMACS Tubing Set TS (REF 161-01), CliniMACS PBS/EDTA Buffer (REF 700-25)
- If the labeling volume (see "Volume adjustment") must be adjusted to 190 mL, 2 vials of CliniMACS CD271 Reagent must be used.

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

Labeling and preparation of bags

- Label bags as: **Cell Collection**, **Bone Marrow Aspirate**, **Cell Preparation, Plasma Waste**, **Wash Waste**.
- Determine the weight of the empty Cell Collection and Cell Preparation Bag.

III. Bone Marrow Aspirate (BMA)

Analysis of BMA

Filtration of BMA and transfer into Cell Preparation Bag

Dilution of BMA

Add buffer: Weight of buffer to be added = Weight of BMA × 4

Centrifugation

300×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

Volume adjustment

Labeling Volume: **95 g** (or 190 g)

- Remove supernatant to adjust the sample using the following equation:
  Weight of supernatant to be removed = Weight of diluted BMA – 95 g
- Remove the supernatant taking care not to resuspend the cell pellet. **Stop the removal of supernatant before losing cells.**
  If the recommended labeling volume exceeds 95 g, adjust the labeling volume to **190 g** using the following equation:
  Weight of buffer to be added = 190 g – Weight of diluted BMA
- Resuspend cell pellet.

IV. Magnetic labeling of the cells

Incubation with CliniMACS® CD271 Reagent

Add **1 vial** of CliniMACS CD271 Reagent if the labeling volume was adjusted to 95 mL or **2 vials** if the labeling volume is 190 mL, incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent:

- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Adjust sample loading volume to 100 g.
  (Adjust the volume if the weight of the BMA is less than 100 g. The volume may range between 100 g and 275 g but must not exceed 275 g).

37000
ch15/15
(Issued: 2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

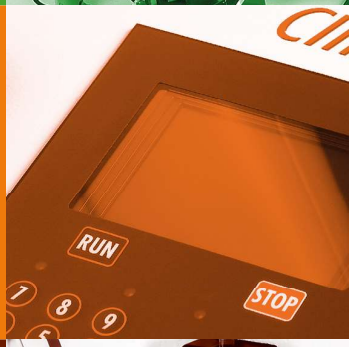
Enrichment of CD271 positive cells (Bone Marrow)

Four STEPS to your target cells

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program ENRICHMENT 3.2



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Cell Collection Bag

Attach Priming Waste Bag and insert pre-column

Insert separation column and load valve no. 5

Load valves nos. 1, 2, 3, and 4

Load pump tubing

Load valves nos. 7 and 8

Load valves nos. 6, 9, 10, and 11

Recheck all tubing and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment of CD62L positive cells

Four STEPS to your target cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the general information, warnings and precautions in the CliniMACS® User Manual:
General information, CliniMACS® CD62L Reagent, Handling of biohazardous material, Cellular starting product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer.

II. First actions required

Material

Up to 4×10^9 CD62L positive cells out of 10×10^9 total nucleated cells:
1 vial of CliniMACS CD62L Reagent (REF 701-19), 1 CliniMACS Tubing Set LS (REF 162-01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v).

- **For the whole preparation procedure use pre-cooled buffer at +2 °C to +8 °C.** Pre-cool the buffer before starting the cell preparation procedure.

Labeling and preparation of bags

- Label bags as: **Cell Collection**, **Cell Preparation, Plasma Waste**, and **Wash Waste**.
- Determine the weight of the empty Cell Collection and Cell Preparation Bag.

III. Leukapheresis product (LP)

Analysis of LP

Transfer of LP into Cell Preparation Bag

Dilution of LP

Add buffer: **Weight of buffer to be added = 600 g – Weight of LP**

Centrifugation

300×g, 15 min, without brake, room temperature (RT: +19 °C to +25 °C).

Volume adjustment

Labeling volume: **95 g**

- Remove the supernatant completely, taking care not to resuspend the cell pellet during the removal of supernatant.
- Resuspend cell pellet carefully after removal of supernatant.
- Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation:
  Target weight of filled CPB = 95 g + Weight of empty CPB
- Resuspend cell pellet.

IV. Magnetic labeling of the cells

Incubation with CliniMACS® CD62L Reagent

Add **1 vial** of CliniMACS CD62L Reagent, incubate on orbital rotator (25 rpm) for 15 min at RT.

Removal of excess reagent:

- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant.
- Resuspend cell pellet.
- Adjust Sample loading volume to **100 g**

37000
ch15/15
(Issued: 2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Enrichment of CD62L positive cells

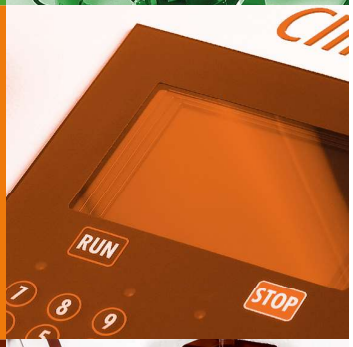
Four STEPS to your target cells

STEP 2
Start the CliniMACS® plus Instrument

Switch-on of the CliniMACS® plus Instrument

Choice of separation program ENRICHMENT 1.1

Sample parameter input



STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Cell Collection Bag

Attach Priming Waste Bag and insert pre-column

Insert separation column and load valve no. 5

Load valves nos. 1, 2, 3, and 4

Load pump tubing

Load valves nos. 7 and 8

Load valves nos. 6, 9, 10, and 11

Recheck all tubing and attachments

Seating of valves

Attach CliniMACS® PBS/EDTA Buffer

Start priming

Check during the priming

Final check of all tubing and attachments

Integrity test

Connect Cell Preparation Bag

Final check of the liquid sensor

STEP 4
CliniMACS® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37000
ch15/15
(Issued:
2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® CD34 Reagent

I. General information, warnings and precautions

General information

The CliniMACS® Plus CD34 System including the CliniMACS ^{plus} Instrument, the CliniMACS CD34 Reagent, the CliniMACS Tubing Set TS or CliniMACS Tubing Set LS, and CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human CD34 positive hematopoietic progenitor cells from heterogeneous hematologic cell populations.

The CD34 antigen is a highly glycosylated 115 kD type 1 integral membrane protein of unknown function which is expressed on 1% to 4% of normal bone marrow cells and less than 0.2% of normal peripheral blood leukocytes, on subsets of bone marrow stromal cells, and on small vessel endothelium of various tissues.

The CliniMACS Plus CD34 System uses selective CD34 monoclonal antibodies conjugated to super-paramagnetic particles. The CD34 positive cells are specifically labeled by incubation with the CliniMACS CD34 Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the enrichment in an automated continuous flow separation process.

The CliniMACS Plus CD34 System passes the antibody-labeled suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled CD34 positive cells (target cells) while the non-labeled cells (non-target cells) flow through the column. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged, and the magnetic field is removed. The target cells are collected in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, cellular starting product, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® CD34 Reagent

Intended purpose

- The CliniMACS® CD34 Reagent is intended for the *in vitro* enrichment of human CD34 positive hematopoietic progenitor cells from heterogeneous hematologic cell populations. in combination with the CliniMACS System only.

- The CliniMACS Plus CD34 System is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS CD34 Reagent, the CliniMACS Tubing Set TS for the "normal scale application" or the CliniMACS Tubing Set LS for the "large scale application", and CliniMACS PBS/EDTA Buffer.

Composition

Each CliniMACS CD34 Reagent vial contains 7.5 mL of a colloidal solution of iron-dextran beads conjugated to murine monoclonal CD34 antibody in PBS/EDTA buffer stabilized with Poloxamer 188 (0.03% w/v).

Side-effects

- When the enriched CD34 positive cells are infused or injected into patients, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndromes. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease appear to be highly susceptible to iron-dextran reactions.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Precautions

- Before use in human applications, the suitability of the target cells must be demonstrated regarding indication, quality and quantity.
- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively within the responsibility of the user.
- The enrichment of CD34 positive cells using the CliniMACS CD34 Reagent must be performed by trained operators only.
- Operators using the CliniMACS CD34 Reagent should have experience in the separation of cells from bone marrow or peripheral blood.
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- The CliniMACS CD34 Reagent is for *in vitro* use only. Do not inject or infuse the reagent directly into the patient. Not for parenteral application.
- The reagent is not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The reagent is for single use only (see fig. 4.1-1).
- Do not use the reagent after the use-by date printed on the vial label (see fig. 4.1-2).
- Do not use the reagent if package is damaged (see fig. 4.1-3). Use undamaged and sealed vials only.
- Store at +2 °C to +8 °C (see fig. 4.1-4). Do not freeze.

Further information

- The CliniMACS CD34 Reagent is tested for endotoxins.
- The reagent is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

Important

This protocol requires the use of components which are not part of the CliniMACS Plus CD34 System (e.g., HSA). Materials of pharmaceutical grade must be used or all risks arising from these materials must be evaluated by the user.

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

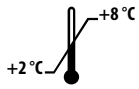


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Fig. 4.1-5: Sterilized using aseptic processing techniques.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

II. First actions required

CliniMACS® Materials required

Note

The application capacity for the enrichment of CD34 positive cells using the CliniMACS Plus CD34 System and one vial of CliniMACS CD34 Reagent is 0.6×10^9 CD34 positive cells out of a total cell number not exceeding 60×10^9 cells. For the enrichment of up to 1.2×10^9 CD34 positive cells out of a total cell number of 120×10^9 cells (large scale preparation), two vials of the CliniMACS CD34 Reagent are needed.

- **CliniMACS® CD34 Reagent, REF 171-01**
  Using the CliniMACS Plus CD34 System, one vial is required for the normal scale application and two vials for the large scale application.

- **CliniMACS Tubing Set TS, REF 161-01**
  One CliniMACS Tubing Set TS must be used for the normal scale application.
  o r
  CliniMACS Tubing Set LS, REF 162-01
  One CliniMACS Tubing Set LS must be used for the large scale application.

- **CliniMACS PBS/EDTA Buffer, REF 700-25**
  CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. For the preparation procedure, two liters of buffer are required. For the separation, one liter of buffer is required. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v). Only FDA-licensed HSA should be used.

Additional materials required

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 “Additional materials required”.

- **Cell Preparation Bag:**
  One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure.

- **Plasma Waste Bag and Wash Waste Bags:**
  Three 600 mL transfer bags, suitable for centrifugation.

- **Cell Collection Bag:**
  One 150 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set.

- Pre-system filter.

- Locking forceps.

- Appropriate syringes (1 mL, 10 mL, 20 mL) and hypodermic 20 gauge needles.

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).

- Sample tubes.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3: "Equipment required".

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement three liters of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v), i.e., add 5 g HSA per liter buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

A master copy of a worksheet is provided with the user manual to record important information and data generated during sample handling and preparation.

- Label one 150 mL transfer bag as: **Cell Collection**. (This should include patient identification, date and time of run, and operator identification.) Insert a Luer/Spike Interconnector into the port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight on the worksheet (C_5).

- Label one 600 mL transfer bag as: **Cell Preparation**. (This should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight on the worksheet (A_2).

- Label one 600 mL transfer bag as: **Plasma Waste**. (This should include patient identification, date, time of run and operator identification.)

- Label two 600 mL transfer bag as: **Wash Waste No. 1** and **Wash Waste No. 2**. (This should include patient identification, date and time of run, and operator identification.)

Important

- Note that **HSA is not a component of the CliniMACS Plus System**. Use only FDA-licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.

- Store the buffer for cell preparation at +19 °C to +25 °C. Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the tubing can vary during the preparation procedure be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- All bag handling should be done in a sterile environment (e.g., laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

III. Leukapheresis product

The following sections describe the recommended procedure for the preparation of the leukapheresis product using the Terumo Sterile Connection Device (TSCD). The TSCD is not a component of the CliniMACS Plus System.

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Analysis of leukapheresis product

Before starting the preparation of the leukapheresis product the following parameters must be determined:

- Total number of leukocytes,
- Percentage of CD34 positive cells,
- Total number of CD34 positive cells,
- Viability.

Other tests might be required depending on the intended use of the cells (e.g. T cell enumeration) or according to individual laboratory practice. Record all data on the worksheet.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Transfer of leukapheresis product into Cell Preparation Bag

1. Record the date and the start time on the worksheet before beginning to prepare the leukapheresis product (A_1).

2. Determine the volume of the original leukapheresis product by estimating 1 mL of leukapheresis product as equivalent to 1 g (1 g = 1 mL).

3. Holding the leukapheresis product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the Cell Preparation Bag to the original leukapheresis product bag.

5. Open the locking forceps to transfer the leukapheresis product material into the Cell Preparation Bag. Use a tubing stripper to clear the tubing from any remaining blood. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see fig. 4.1-6). Retain the original leukapheresis bag until cell separation and final cell analysis have been completed.

7. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the leukapheresis product. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS PRODUCT (This should include patient identification.) and retain for cell analysis Minimally, analysis of the WBC content, CD34 enumeration and viability testing are recommended.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight on the worksheet (A_3).

9. Determine the actual weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the Cell Preparation Bag filled with leukapheresis product (A_3). Record the weight on the worksheet (A_4).

10. If the weight of the leukapheresis product (A_4) is more than 200 g, but the number of cells is less than 120×10^9 total cells and 1.2×10^9 CD34 positive cells, centrifuge the sample to reduce the volume to 200 g at the most and proceed with "Dilution of leukapheresis product". Note the reduced volume on the worksheet ($A_{4\ reduced}$).

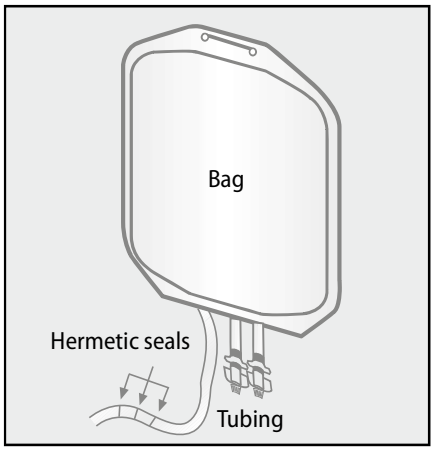


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

Worksheet equation:
$A_4 = A_3 - A_2$

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Dilution of leukapheresis product

The leukapheresis product must be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Calculate the amount of buffer to be added using the following equation and record it on the worksheet (A_5).

Worksheet equation: $A_5 = A_4 \times 2$

Weight of buffer to be added (g) (A_5)	=	Weight of leukapheresis product (g) (A_4)	×	2

1. Take a plasma transfer set and ensure the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer (A_5) to the Cell Preparation Bag.

5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the actual amount of buffer added on the worksheet (A_6).

6. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight on the worksheet (A_7).

Worksheet equation: $A_8 = A_7 - A_2$

9. Determine the weight of diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the filled Cell Preparation Bag (A_7). Record the weight on the worksheet (A_8).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C). Record the centrifugation conditions on the worksheet (A_9).

5. Remove the bag from the centrifuge, taking care not to resuspend the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Volume adjustment

1. For magnetic labeling of CD34 positive cells, the optimal weight of the cell sample is a) 95 g (± 5 g), if one vial of CliniMACS CD34 Reagent is sufficient, or b) 190 g (± 5 g), if two reagent vials are needed (see table 4.1-1).

   Calculate the amount of supernatant to be removed to adjust the sample to 95 g (or 190 g) using the equation below:

   a) Weight of supernatant to be removed (g) = Weight of diluted leukapheresis product (g) – 95 g

   b) Weight of supernatant to be removed (g) = Weight of diluted leukapheresis product (g) – 190 g

   Record the amount of supernatant to be removed on the worksheet (A_{10}).

2. Place the empty Plasma Waste Bag on the balance and tare the balance.

3. Open the locking forceps next to the Cell Preparation Bag. Use the balance display to monitor the weight of the Plasma Waste Bag while removing the supernatant. Using the plasma extractor, carefully press out excess supernatant using the plasma extractor. Continue until the calculated amount of supernatant (A_{10}) has been transferred into the Plasma Waste Bag so that a) 95 g or b) 190 g remain in the Cell Preparation Bag.

	Normal scale preparation	Large scale preparation
Labeled cells	0.6×10^9	$0.6–1.2\times10^9$
Total cells	60×10^9 WBC	$60\text{-}120\times10^9$ WBC
Number of CD34 Reagent vials	1	2
Optimal labeling weight	95 g (± 5 g)	190 g (± 5 g)

Table 4.1-1: Optimal labeling weight for the enrichment of CD34 positive cells

Worksheet equation:
a) $A_{10} = A_8 - 95$ g o r
b) $A_{10} = A_8 - 190$ g

Important

- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is open before beginning the transfer. Release the extractor handle slowly.

- During removal of supernatant be careful not to lose cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

4. When the appropriate amount of supernatant has been transferred, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Record the weight of the supernatant removed on the worksheet (A_{11}).

5. Using the heater sealer, seal off the tubing to disconnect the Plasma Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag.

6. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

7. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight on the worksheet (A_{12}).

8. Determine the weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the filled Cell Preparation Bag (A_{12}). Record the calculated weight on the worksheet (A_{13}).

9. Keep the Plasma Waste Bag until the separation and final analysis of all cells has been accomplished.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

IV. Magnetic labeling of cells

One vial (7.5 mL) of CliniMACS CD34 Reagent is ready to use and sufficient for one normal scale preparation as described below. For a large scale preparation, two vials of CliniMACS CD34 Reagent are required. The reagent is not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze. The reagent is to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent are printed on the vial. DO NOT use the reagent after the use-by date.

Incubation with the CliniMACS® CD34 Reagent

1. Record the lot number (B_1) and use-by date (B_2) of the CliniMACS® CD34 Reagent on the worksheet.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from a) one vial or b) two reagent vials (7.5 mL each). A 10 mL syringe is sufficient to remove the contents of one vial, or respectively, a 20 mL syringe is sufficient to remove the contents of two vials of reagent for a large scale application. The syringe should be equipped with a 20 gauge needle.

3. Using the injection port on the sampling site coupler, inject the entire volume of reagent into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time on the worksheet (B_3).

5. Place the Cell Preparation Bag flat on the orbital rotator set to a speed of approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time on the worksheet (B_4).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Removal of excess reagent

Wash no. 1

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least one liter of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer (i.e. add 400 g to 500 g of buffer). To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of buffer transferred into the Cell Preparation Bag on the worksheet (B_5).

5. Close the locking forceps next to the Cell Preparation Bag. Seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag No. 1 to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag No. 1 securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C). Record the centrifugation conditions on the worksheet (B_6).

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

13. Place the Wash Waste Bag No. 1 on the balance. Tare the balance.

14. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much supernatant as possible from the Cell Preparation Bag. Record the amount of removed supernatant on the worksheet (B_7).

15. Close the locking forceps and seal off the tubing to disconnect the Wash Waste Bag No. 1 leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.

16. Keep the Wash Waste Bag No. 1 until the separation and final analysis of all cells has been accomplished.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

Wash no. 2

18. Using the TSCD, connect the plasma transfer set, inserted into a buffer bag containing at least 500 mL of buffer, to the Cell Preparation Bag. Confirm the clamp on the plasma transfer set is closed.

19. Place the Cell Preparation Bag on the balance. Hang the buffer bag on the bag hanger. Tare the balance.

20. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer (i.e., add approximately 500 g of buffer). To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of buffer transferred into the Cell Preparation Bag on the worksheet (B_8).

21. Close the locking forceps next to the Cell Preparation Bag. Seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

22. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

23. Using the TSCD, connect the empty Wash Waste Bag No. 2 to the Cell Preparation Bag.

Important

- For the normal scale preparation (one vial of CliniMACS CD34 Reagent), the volume of supernatant removed should be at least 500 mL.
- For the large scale preparation (two vials of CliniMACS CD34 Reagent), the volume of supernatant removed should be at least 450 mL.
- If the supernatant removed is less than the value listed above a total of three washing steps (instead of only two) is recommended. Otherwise the removal of unbound reagent may be insufficient.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

24. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag No. 2 securely to the centrifuge bucket.

25. Centrifuge at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C). Record the centrifugation conditions on the worksheet (B_9).

26. Taking care not to disturb the cell pellet, remove the bags from the centrifuge. Carefully hang the Cell Preparation Bag on the plasma extractor.

27. Place the Wash Waste Bag No. 2 on the balance. Tare the balance.

28. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much supernatant as possible from the Cell Preparation Bag. Record the amount of removed supernatant on the worksheet (B_{10}).

29. Close the locking forceps and seal off the tubing to disconnect the Wash Waste Bag No. 2.

30. Keep the Wash Waste Bag No. 2 until the separation and final analysis of all cells has been accomplished.

31. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

Worksheet equation:
$B_{12} = B_{11} - A_2$

32. Weigh the Cell Preparation Bag and record the weight on the worksheet (B_{11}). Determine the weight of the leukapheresis product after the washes by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the filled Cell Preparation Bag (B_{11}). Record the weight on the worksheet (B_{12}).

Worksheet equation:
a) $B_{13} = 150\ g - B_{12}$ or
b) $B_{13} = 275\ g - B_{12}$

33. Adjust sample loading volume: Calculate the amount of buffer necessary to adjust the weight of the cell suspension to approximately a) 150 g (normal scale preparation) or b) 275 g (large scale preparation), (B_{13}).

34. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

35. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

36. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer (B_{13}) to the Cell Preparation Bag.

37. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

38. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

39. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (This should include patient identification.) and retain for cell analysis.

40. Weigh the Cell Preparation Bag and record the weight on the worksheet (B_{14}). Determine the weight of the leukapheresis product (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the filled Cell Preparation Bag (B_{14}). Record the weight on the worksheet (B_{15}).

Worksheet equation:
$B_{15} = B_{14} - A_2$

Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Worksheet for preparation and enrichment of CD34 positive cells

Patient Data _____

_____

Operator ID _____

Transfer of leukapheresis product into Cell Preparation Bag

Starting: Date _____ Time _____ (A_1)

Remove leukapheresis product sample ☐ (if yes ✓)

Weight of empty Cell Preparation Bag *(with locking forceps and sampling site coupler)* _____ g (A_2)

Weight of Cell Preparation Bag filled with leukapheresis product _____ g (A_3)

Weight of leukapheresis product (difference between filled and empty Cell Preparation Bag)
$A_4 = A_3 - A_2$
Should not exceed 200 g. _____ g (A_4)

Weight of leukapheresis product after volume reduction A4 reduced (difference between filled bag and empty Cell Preparation Bag, should not exceed 200 g, see below) _____ g ($A_{4\ reduced}$)

Dilution of leukapheresis product

The leukapheresis product should be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Calculate the amount of buffer to be added using the equation below.

Weight of buffer to be added	=	Weight of leukapheresis product	×	2

Weight of buffer to be added
$A_5 = A_4 \times 2$ _____ g (A_5)

Actual amount of buffer added _____ g (A_6)

Weight of filled Cell Preparation Bag after dilution _____ g (A_7)

Weight of diluted leukapheresis product
$A_8 = A_7 - A_2$ _____ g (A_8)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Adjusting the leukapheresis product to 95 g or 190 g

The optimal weight of the leukapheresis product for magnetic labeling of the CD34 positive cells depends on the number of CliniMACS CD34 Reagent vials to be used for labeling. The contents of one CliniMACS CD34 Reagent vial is capable of labeling up to 0.6×10^9 CD34 positive cells out of a total leukocyte number of up to 60×10^9 cells. In this case the optimal labeling weight is 95 g. For large scale preparations (cell numbers in the range of 0.6 to 1.2×10^9 CD34 positive cells out of $60–120\times10^9$ total cells) use two vials of CliniMACS CD34 Reagent and adjust the labeling weight to 190 g. After centrifugation follow (a) or (b) respectively.

Centrifugation conditions
Time:
g Force:
Temperature:

(A_9)

a) Calculate the amount of supernatant to be removed to give a final volume of 95 g. Use equation below.

Amount of supernatant to be removed	=	Weight of diluted leukapheresis product	–	95 g

Amount of supernatant to be removed
$A_{10} = A_8 - 95$ g _____ g (A_{10})

Actual amount of supernatant removed _____ g (A_{11})

Weight of filled Cell Preparation Bag after volume adjustment _____ g (A_{12})

Weight of leukapheresis product after volume adjustment
$A_{13} = A_{12} - A_2$ _____ g (A_{13})

b) Calculate the amount of supernatant to be removed to give a final volume of 190 g. Use equation below.

Amount of supernatant to be removed	=	Weight of diluted leukapheresis product	–	190 g

Amount of supernatant to be removed
$A_{10} = A_8 - 190$ g _____ g (A_{10})

Actual amount of supernatant removed _____ g (A_{11})

Weight of filled Cell Preparation Bag after volume adjustment _____ g (A_{12})

Weight of leukapheresis product after volume adjustment
$A_{13} = A_{12} - A_2$ _____ g (A_{13})

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Magnetic labeling of CD34 positive cells

Lot number of CD34 Reagent _____ (B_1)

Second CD34 Reagent (optional) _____ (B_1)

Use-by date of CD34 Reagent _____ (B_2)

Second CD34 Reagent (optional) _____ (B_2)

Incubation

Incubation start time _____ (B_3)

Incubation stop time _____ (B_4)

Wash no. 1

Amount of buffer transferred into the Cell Preparation Bag _____ g (B_5)

Centrifugation conditions
Time:
g Force:
Temperature:

(B_6)

Amount of removed supernatant _____ g (B_7)

Wash no. 2

Amount of buffer transferred into the Cell Preparation Bag _____ g (B_8)

Centrifugation conditions
Time:
g Force:
Temperature:

(B_9)

Amount of removed supernatant _____ g (B_{10})

Weight of filled Cell Preparation Bag after washing procedure _____ g (B_{11})

Weight of leukapheresis product after washing procedure
$B_{12} = B_{11} - A_2$ _____ g (B_{12})

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Final sample preparation

Adjust the volume of the leukapheresis product for loading on the CliniMACS plus Instrument to 150 g (normal scale preparation) or 275 g (large scale preparation) - sample loading volume. Calculate the amount of buffer to be added:

Amount of buffer to be added
a) B_{13} = 150 g – B_{12} (normal scale)
b) B_{13} = 275 g – B_{12} (large scale) _____ g (B_{13})

Optional:
Remove leukapheresis
product sample (**ORIGINAL**) ☐ (if yes ✓)

Weight of filled Cell Preparation Bag
after buffer addition _____ g (B_{14})

Weight of leukapheresis product
after buffer addition (sample loading volume)
B_{15} = B_{14} – A_2 _____ g (B_{15})

CliniMACS® Separation (Run)

Date, time started run: Date _____ Time _____ (C_1)

Lot number of tubing set _____ (C_2)

Use-by date of tubing set _____ (C_3)

Calculate the amount of the target cell fraction

Weight of filled Cell Collection Bag
after the run has been finished _____ g (C_4)

Weight of empty Cell Collection Bag
(with locking forceps) _____ g (C_5)

Weight of target cell fraction
C_6 = C_4 – C_5 _____ g (C_6)

Process code _____ (C_7)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Analysis data

This list is an example of analysis data acquired. Further analysis of additional parameters should be included based on the intended use of the target cells.

Leukapheresis product
(before preparation procedure, sample LEUKAPHERESIS PRODUCT)

Total number of leukocytes _____

Viability _____

Total number of CD34 positive cells _____

Percentage of CD34 positive cells _____

Leukapheresis product
(after magnetic labeling, sample ORIGINAL)

Total number of leukocytes _____

Viability _____

Total number of CD34 positive cells _____

Target cell fraction of CD34 positive cells
(Cell Collection Bag)

Total number of leukocytes _____

Viability _____

Total number of CD34 positive cells _____

Purity of CD34 positive cells _____

Recovery of CD34 positive cells _____

Non-target cell fraction
(Negative Fraction Bag)

Total number of leukocytes _____

Viability _____

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® CD133 Reagent (Leukapheresis Product)

I. General information, warnings and precautions

General information

The CliniMACS® Plus CD133 System including the CliniMACS ^{plus} Instrument, the CliniMACS CD133 Reagent, the CliniMACS Tubing Set TS or CliniMACS Tubing Set LS, and CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human CD133 positive progenitor cells from heterogeneous hematologic cell populations. For bone marrow aspirate as starting material, see chapter STEP 1 "CliniMACS CD133 Reagent (Bone Marrow)".

The CD133 antigen is specifically expressed on an immature subset of CD34 positive cells. The molecule is a 5-transmembrane cell surface antigen with a molecular weight of 117 KD and is expressed on 30-60% of all CD34 positive cells including CD34 bright cells. CD133 is expressed on lineage non-committed stem and progenitor cells.

The CliniMACS Plus CD133 System uses selective CD133 monoclonal antibodies conjugated to super-paramagnetic particles. The CD133 positive cells are specifically labeled by incubation with the CliniMACS CD133 Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the enrichment in an automated continuous flow separation process.

The CliniMACS Plus CD133 System passes the antibody-labeled suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled CD133 positive cells (target cells) while the non-labeled cells (non-target cells) flow through the column. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged, and the magnetic field is removed. The target cells are collected in the Cell Collection Bag.

Important

- For bone marrow aspirate as starting material, please refer to chapter 4 – STEP 1 "CliniMACS CD133 Reagent (Bone marrow)".

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, cellular starting product, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® CD133 Reagent

Intended purpose

- The CliniMACS® CD133 Reagent is intended for the *in vitro* enrichment of human CD133 positive progenitor cells from heterogeneous hematologic cell populations in combination with the CliniMACS System only.

- The CliniMACS Plus CD133 (LP) System is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS CD133 Reagent, the CliniMACS Tubing Set TS, for the "normal scale application" or the CliniMACS Tubing Set LS for the "large scale application", and CliniMACS PBS/EDTA Buffer.

Composition

Each CliniMACS CD133 Reagent vial contains 7.5 mL of a colloidal solution of iron-dextran beads conjugated to murine monoclonal CD133 antibody in PBS/EDTA buffer stabilized with Poloxamer 188 (0.03% w/v).

Side-effects

- When the enriched CD133 positive cells are infused into patients, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndromes. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease appear to be highly susceptible to iron-dextran reactions.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Precautions

- Before use in human applications, the suitability of the target cells must be demonstrated regarding indication, quality and quantity.
- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively the responsibility of the user.
- The enrichment of CD133 positive cells using the CliniMACS CD133 Reagent must be performed by trained operators only.
- Operators using the CliniMACS CD133 Reagent should have experience in the separation of cells from bone marrow or peripheral blood.
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- The CliniMACS CD133 Reagent is for *in vitro* use only. Do not inject or infuse the reagent directly into the patient. Not for parenteral application.
- The reagent is not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The reagent is for single use only (see fig. 4.1-1).
- Do not use the reagent after the use-by date printed on the vial label (see fig. 4.1-2).
- Do not use the reagent if package is damaged (see fig. 4.1-3). Use undamaged and sealed vials only.
- Store at +2 °C to +8 °C (see fig. 4.1-4). Do not freeze.

Further information

- The CliniMACS CD133 Reagent is tested for endotoxins.
- The reagent is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

Important

This protocol requires the use of components which are not part of the CliniMACS Plus CD133 System (e.g., HSA). Therefore all materials used must be pharmaceutical grade or FDA-licensed for human use.

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

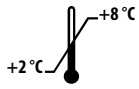


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Fig. 4.1-5: Sterilized using aseptic processing techniques.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

II. First actions required

CliniMACS® Materials required

Note

The application capacity for the enrichment of CD133 positive cells using the CliniMACS Plus CD133 System and one vial of CliniMACS CD133 Reagent is 0.6×10^9 CD133 positive cells out of a total cell number not exceeding 60×10^9 (normal scale application). For the enrichment of up to 1.2×10^9 CD133 positive cells out of a total cell number of 120×10^9 (large scale application), two vials of the CliniMACS CD133 Reagent are needed.

- **CliniMACS® CD133 Reagent, REF 172-01**
  Using the CliniMACS Plus CD133 System, one vial is required for the normal scale application and two vials for the large scale application.

- **CliniMACS Tubing Set TS, REF 161-01**
  One CliniMACS Tubing Set TS must be used for the normal scale application.
  o r
  CliniMACS Tubing Set LS, REF 162-01
  One CliniMACS Tubing Set LS must be used for the large scale application.

- **CliniMACS PBS/EDTA Buffer, REF 700-25**
  CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. For the preparation procedure, two liters of buffer are required. For the separation, one liter of buffer is required. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v). Only FDA-licensed HSA should be used.

Additional materials required

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 "Additional materials required".

- *Cell Preparation Bag:*
  One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure.

- *Plasma Waste Bag and Wash Waste Bags:*
  Three 600 mL transfer bags, suitable for centrifugation.

- *Cell Collection Bag:*
  One 150 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set.

- Pre-system filter.

- Locking forceps.

- Appropriate syringes (1 mL, 10 mL, 20 mL) and hypodermic 20 gauge needles.

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).

- Sample tubes.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Equipment required

Please find information regarding additional equipment required for a CliniMACS Plus Separation in chapter 3: "Equipment required".

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement three liters of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v), i.e., add 5 g HSA per liter buffer. Use only FDA licensed HSA.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

A master copy of a worksheet is provided with the user manual to record important information and data generated during sample handling and preparation.

- Label one 150 mL transfer bag as: **Cell Collection**. (This should include patient identification, date and time of run, and operator identification.) Insert a Luer/Spike Interconnector into the port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight on the worksheet (C5).

- Label one 600 mL transfer bag as: **Cell Preparation**. (This should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight on the worksheet (A_2).

- Label one 600 mL transfer bag as: **Plasma Waste**. (This should include patient identification, date, time of run and operator identification.)

- Label two 600 mL transfer bag as: **Wash Waste No. 1** and **Wash Waste No. 2**. (This should include patient identification, date and time of run, operator identification.)

Important

- Note that **HSA is not a component of the CliniMACS Plus System.** Use only FDA-licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.

- Store the buffer for cell preparation at +19 °C to +25 °C. Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the tubing can vary during the preparation procedure, be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- All bag handling should be performed in a sterile environment (e.g., laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

III. Leukapheresis product

The following sections describe the recommended procedure for the preparation of the leukapheresis product using the Terumo Sterile Connection Device (TSCD). The TSCD is not a component of the CliniMACS Plus System.

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all necessary supplies and equipment are available.

Analysis of leukapheresis product

Before starting the preparation of the leukapheresis product the following parameters must be determined:

- Total number of leukocytes,
- Percentage of CD133 positive cells,
- Total number of CD133 positive cells,
- Viability.

Other tests might be required depending on the intended use of the cells (e.g. T cell enumeration) or according to individual laboratory practice. Record all data on the worksheet.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Transfer of leukapheresis product into Cell Preparation Bag

1. Record the date and the start time on the worksheet before beginning to prepare the leukapheresis product (A_1).

2. Determine the volume of the original leukapheresis product by estimating 1 mL of leukapheresis product as equivalent to 1 g (1 g = 1 mL).

3. Holding the leukapheresis product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the Cell Preparation Bag to the original leukapheresis product bag.

5. Open the locking forceps to transfer the leukapheresis product into the Cell Preparation Bag. Use a tubing stripper to clear the tubing from any remaining blood. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see fig. 4.1-6). Retain the original leukapheresis bag until cell separation and final cell analysis have been completed.

7. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the leukapheresis product. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS PRODUCT (This should include patient identification.) and retain for cell analysis. Minimally, analysis of the WBC content, CD133 enumeration and viability testing are recommended.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight on the worksheet (A_3).

9. Determine the actual weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the Cell Preparation Bag filled with leukapheresis product (A_3). Record the weight on the worksheet (A_4).

10. If the weight of the leukapheresis product (A_4) is more than 200 g, but the number of cells is less than 120×10^9 total cells and 1.2×10^9 CD133 positive cells, centrifuge the sample to reduce the volume to 200 g at the most and proceed with "Dilution of leukapheresis product". Record the reduced volume on the worksheet (A_4 reduced).

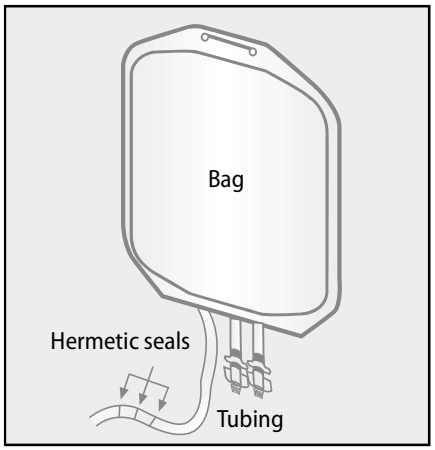


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

Worksheet equation:
$A_4 = A_3 - A_2$

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Dilution of leukapheresis product

The leukapheresis product must be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Calculate the amount of buffer to be added using the following equation and record it on the worksheet (A_5).

Worksheet equation: $A_5 = A_4 \times 2$

Weight of buffer to be added (g)	=	Weight of leukapheresis product (g)	×	2

1. Take a plasma transfer set and ensure the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer (A_5) to the Cell Preparation Bag.

5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the weight of buffer added on the worksheet (A_6).

6. Using the heat sealer, make three hermetic seals between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight on the worksheet (A_7).

Worksheet equation: $A_8 = A_7 - A_2$

9. Determine the weight of diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the filled Cell Preparation Bag (A_7). Record the weight on the worksheet (A_8).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C). Record the centrifugation conditions on the worksheet (A_9).

5. Remove the bag from the centrifuge, taking care not to resuspend the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Volume adjustment

1. For magnetic labeling of CD133 positive cells, the optimal weight of the cell sample is a) 95 g (± 5 g), if one vial of CliniMACS CD133 Reagent is sufficient, or b) 190 g (± 5 g), if two reagent vials are needed (see table 4.1-1).

   Calculate the amount of supernatant to be removed to adjust the sample to 95 g (or 190 g) using the equation below:

   a) Weight of supernatant to be removed (g) = Weight of diluted leukapheresis product (g) – 95 g

   b) Weight of supernatant to be removed (g) = Weight of diluted leukapheresis product (g) – 190 g

   Record the amount of supernatant to be removed on the worksheet (A_{10}).

2. Place the empty Plasma Waste Bag on the balance and tare the balance.

3. Open the locking forceps next to the Cell Preparation Bag. Use the balance display to monitor the weight of the Plasma Waste Bag while removing the supernatant. Using the plasma extractor, carefully press out excess supernatant until the calculated "weight of supernatant to be removed" (A_{10}) has been transferred into the Plasma Waste Bag so that a) 95 g or b) 190 g remain in the Cell Preparation Bag.

	Normal scale application	Large scale application
Labeled cells	0.6×10^9	$0.6–1.2\times10^9$
Total cells	60×10^9 WBC	$60\text{-}120\times10^9$ WBC
Number of CD133 Reagent vials	1	2
Optimal labeling weight	95 g (± 5 g)	190 g (± 5 g)

Table 4.1-1: Optimal labeling weight for the enrichment of CD133 positive cells

Worksheet equation:

a) $A_{10} = A_8 - 95$ g or

b) $A_{10} = A_8 - 190$ g

Important

- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is open before beginning the transfer. Release the extractor handle slowly.

- During removal of supernatant be careful not to lose cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

4. When the appropriate amount of supernatant has been transferred, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Record the weight of the supernatant removed on the worksheet (A_{11}).

5. Using the heater sealer, seal off the tubing to disconnect the Plasma Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag.

6. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight on the worksheet (A_{12}).

7. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

8. Determine the weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the filled Cell Preparation Bag (A_{12}). Record the calculated weight on the worksheet (A_{13}).

9. Keep the Plasma Waste Bag until the separation and final analysis of all cells has been completed.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

IV. Magnetic labeling of cells

One vial (7.5 mL) of CliniMACS CD133 Reagent is ready to use and sufficient for one normal scale application as described below. For a large scale application, two vials of CliniMACS CD133 Reagent are required. The reagent is not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze. The reagent is to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent are printed on the vial. DO NOT use the reagent after the use-by date.

Incubation with the CliniMACS® CD133 Reagent

1. Record the lot number (B_1) and use-by date (B_2) of the CliniMACS® CD133 Reagent on the worksheet.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from a) one vial or b) two reagent vials (7.5 mL each). A 10 mL syringe is sufficient to remove the contents of one vial, or respectively, a 20 mL syringe is sufficient to remove the contents of two vials of reagent for a large scale application. The syringe used should be equipped with a 20 gauge needle.

3. Using the injection port on the sampling site coupler, inject the entire volume of reagent into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time on the worksheet (B_3).

5. Place the Cell Preparation Bag flat on the orbital rotator is set to a speed of approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time on the worksheet (B_4).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Removal of excess reagent

Wash no. 1

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least one liter of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer (i.e. add 400 g to 500 g of buffer). To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of buffer transferred into the Cell Preparation Bag on the worksheet (B_5).

5. Close the locking forceps next to the Cell Preparation Bag. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag No. 1 to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag No. 1 securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 200×g for 15 minutes (without brake) at room temperature (+19 °C to +25 °C). Note the centrifugation conditions on the worksheet (B_6).

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag No. 1 on the balance. Tare the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

14. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much excess supernatant as possible from the Cell Preparation Bag. Record the amount of removed supernatant on the worksheet (B_7).

15. Close the locking forceps and using the heat sealer, seal off the tubing to disconnect the Wash Waste Bag No. 1 leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.

16. Keep the Wash Waste Bag No.1 until the separation and final analysis of all cells has been accomplished.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

Wash no. 2

18. Using the TSCD, connect the plasma transfer set, inserted into a buffer bag containing at least 500 mL of buffer, to the Cell Preparation Bag. Confirm the clamp on the plasma transfer set is closed.

19. Place the Cell Preparation Bag on the balance. Hang the buffer bag on the bag hanger. Tare the balance.

20. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer (i.e., add approximately 500 g of buffer). To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of buffer transferred into the Cell Preparation Bag on the worksheet (B_8).

21. Close the locking forceps next to the Cell Preparation Bag. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

22. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

23. Using the TSCD, connect the empty Wash Waste Bag No. 2 to the Cell Preparation Bag.

24. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag No. 2 securely to the centrifuge bucket.

Important

- For the normal scale application (one vial of CliniMACS CD133 Reagent), the volume of supernatant removed should be at least 500 mL.
- For the large scale application (two vials of CliniMACS CD133 Reagent), the volume of supernatant removed should be at least 450 mL.
- If the supernatant removed is less than the value listed above a total of three washing steps (instead of only two) is recommended. Otherwise the removal of unbound reagent may be insufficient.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

25. Centrifuge at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C). Record the centrifugation conditions on the worksheet (B_9).

26. Taking care not to disturb the cell pellet, remove the bags from the centrifuge. Carefully hang the Cell Preparation Bag on the plasma extractor.

27. Place the Wash Waste Bag No. 2 on the balance. Tare the balance.

28. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much excess supernatant as possible from the Cell Preparation Bag. Record the amount of removed supernatant on the worksheet (B_{10}).

29. Close the locking forceps and seal off the tubing to disconnect the Wash Waste Bag No. 2.

30. Keep the Wash Waste Bag No. 2 until the separation and final analysis of all cells has been accomplished.

31. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

Worksheet equation:
$B_{12} = B_{11} - A_2$

32. Weigh the Cell Preparation Bag and record the weight on the worksheet (B_{11}). Determine the weight of the leukapheresis product after the washes by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the filled Cell Preparation Bag (B_{11}). Record the weight on the worksheet (B_{12}).

Worksheet equation:
a) $B_{13} = 150\ g - B_{12}$ or
b) $B_{13} = 275\ g - B_{12}$

33. Adjust sample loading volume: Calculate the amount of buffer necessary to adjust the weight of the cell suspension to approximately a) 150 g (normal scale application) or b) 275 g (large scale application), (B_{13}).

34. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

35. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

36. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer (B_{13}) to the Cell Preparation Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

37. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

38. Using the heat sealer, seal off the tubing. Disconnect the buffer bag.

39. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (This should include patient identification.) and retain for cell analysis. WBC content, viability testing and CD133 enumeration are recommended.

40. Weigh the Cell Preparation Bag and record the weight on the worksheet (B_{14}). Determine the weight of the leukapheresis product (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the filled Cell Preparation Bag (B_{14}). Record the weight on the worksheet (B_{15}).

Worksheet equation:
$B_{15} = B_{14} - A_2$

Proceed to STEP 2

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Worksheet for preparation and enrichment of CD133 positive cells from leukapheresis product

Patient Data _____

_____

Operator ID _____

Transfer of leukapheresis product into Cell Preparation Bag

Starting: Date _____ Time _____ (A_1)

Remove leukapheresis product sample ☐ (if yes ✓)

Weight of empty Cell Preparation Bag *(with locking forceps and sampling site coupler)* _____ g (A_2)

Weight of Cell Preparation Bag filled with leukapheresis product _____ g (A_3)

Weight of leukapheresis product (difference between filled and empty Cell Preparation Bag)
$A_4 = A_3 - A_2$
Should not exceed 200 g. _____ g (A_4)

Weight of leukapheresis product after volume reduction A4 reduced (difference between filled bag and empty Cell Preparation Bag, should not exceed 200 g, see below) _____ g ($A_{4\ reduced}$)

Dilution of leukapheresis product

The leukapheresis product should be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Calculate the amount of buffer to be added using the equation below.

Weight of buffer to be added	=	Weight of leukapheresis product	×	2

Weight of buffer to be added
$A_5 = A_4 \times 2$ _____ g (A_5)

Actual amount of buffer added _____ g (A_6)

Weight of filled Cell Preparation Bag after dilution _____ g (A_7)

Weight of diluted leukapheresis product
$A_8 = A_7 - A_2$ _____ g (A_8)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Adjusting the leukapheresis product to 95 g or 190 g

The optimal weight of the leukapheresis product for magnetic labeling of the CD133 positive cells depends on the number of CliniMACS CD133 Reagent vials to be used for labeling. The contents of one CliniMACS CD133 Reagent vial is capable of labeling up to 0.6×10^9 CD133 positive cells out of a total leukocyte number of up to 60×10^9 cells. In this case the optimal labeling weight is 95 g. For large scale preparations (cell numbers in the range of 0.6 to 1.2×10^9 CD133 positive cells out of $60–120\times10^9$ total cells) use two vials of CliniMACS CD133 Reagent and adjust the labeling weight to 190 g. After centrifugation follow (a) or (b) respectively.

Centrifugation conditions
Time:
g Force:
Temperature:

(A_9)

a) Calculate the amount of supernatant to be removed to give a final volume of 95 g. Use equation below.

Amount of supernatant to be removed	=	Weight of diluted leukapheresis product	–	95 g

Amount of supernatant to be removed
$A_{10} = A_8 - 95$ g _____ g (A_{10})

Actual amount of supernatant removed _____ g (A_{11})

Weight of filled Cell Preparation Bag after volume adjustment _____ g (A_{12})

Weight of leukapheresis product after volume adjustment
$A_{13} = A_{12} - A_2$ _____ g (A_{13})

b) Calculate the amount of supernatant to be removed to give a final volume of 190 g. Use equation below.

Amount of supernatant to be removed	=	Weight of diluted leukapheresis product	–	190 g

Amount of supernatant to be removed
$A_{10} = A_8 - 190$ g _____ g (A_{10})

Actual amount of supernatant removed _____ g (A_{11})

Weight of filled Cell Preparation Bag after volume adjustment _____ g (A_{12})

Weight of leukapheresis product after volume adjustment
$A_{13} = A_{12} - A_2$ _____ g (A_{13})

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Magnetic labeling of CD133 positive cells

Lot number of CD133 Reagent _____ (B_1)

Use-by date of CD133 Reagent _____ (B_2)

Second CD133 Reagent (optional) _____ (B_1)

Second CD133 Reagent (optional) _____ (B_2)

Incubation

Incubation start time _____ (B_3)

Incubation stop time _____ (B_4)

Wash no. 1

Amount of buffer transferred into the Cell Preparation Bag _____ g (B_5)

Centrifugation conditions
Time:
g Force:
Temperature:

(B_6)

Amount of removed supernatant _____ g (B_7)

Wash no. 2

Amount of buffer transferred into the Cell Preparation Bag _____ g (B_8)

Centrifugation conditions
Time:
g Force:
Temperature:

(B_9)

Amount of removed supernatant _____ g (B_{10})

Weight of filled Cell Preparation Bag after washing procedure _____ g (B_{11})

Weight of leukapheresis product after washing procedure
$B_{12} = B_{11} - A_2$ _____ g (B_{12})

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Final sample preparation

Adjust the volume of the leukapheresis product for loading on the CliniMACS plus Instrument to 150 g (normal scale preparation) or 275 g (large scale preparation) - sample loading volume. Calculate the amount of buffer to be added:

Amount of buffer to be added
a) B_{13} = 150 g – B_{12} (normal scale)
b) B_{13} = 275 g – B_{12} (large scale) _____ g (B_{13})

Optional: Remove leukapheresis product sample (ORIGINAL) ☐ (if yes ✓)

Weight of filled Cell Preparation Bag after buffer addition _____ g (B_{14})

Weight of leukapheresis product after buffer addition (sample loading volume)
B_{15} = B_{14} – A_2 _____ g (B_{15})

CliniMACS® Separation (Run)

Date, time started run: Date _____ Time _____ (C_1)

Lot number of tubing set _____ (C_2)

use-by date of tubing set _____ (C_3)

Calculate the amount of the target cell fraction

Weight of filled Cell Collection Bag after the run has been finished _____ g (C_4)

Weight of empty Cell Collection Bag (with locking forceps) _____ g (C_5)

Weight of target cell fraction
C_6 = C_4 – C_5 _____ g (C_6)

Process code _____ (C_7)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Analysis data

This list is an example of analysis data acquired. Further analysis of additional parameters should be included based on the intended use of the target cells.

Leukapheresis product
(before preparation procedure, sample LEUKAPHERESIS PRODUCT)

Total number of leukocytes _____

Viability _____

Total number of CD133 positive cells _____

Percentage of CD133 positive cells _____

Leukapheresis product
(after magnetic labeling, sample ORIGINAL)

Total number of leukocytes _____

Viability _____

Total number of CD133 positive cells _____

Target cell fraction of CD133 positive cells
(Cell Collection Bag)

Total number of leukocytes _____

Viability _____

Total number of CD133 positive cells _____

Purity of CD133 positive cells _____

Recovery of CD133 positive cells _____

Non-target cell fraction
(Negative Fraction Bag)

Total number of leukocytes _____

Viability _____

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® CD14 Reagent

I. General information, warnings and precautions

General information

The CliniMACS® Plus CD14 System including the CliniMACS plus Instrument, the CliniMACS CD14 Reagent, the CliniMACS Tubing Set TS, and CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human CD14 positive cells from heterogeneous hematologic cell populations.

The CD14 antigen is a 53-55 kD cell membrane glycoprotein expressed on monocytes. The CD14 positive cells may be used for the subsequent generation of immature/mature dendritic cells (DC). The CD14 antigen is known as a receptor for complex of LPS and LPS binding protein (LBP).

The CliniMACS Plus CD14 System uses selective CD14 monoclonal antibodies conjugated to super-paramagnetic particles. The CD14 positive cells are specifically labeled by incubation with the CliniMACS CD14 Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the enrichment in an automated continuous flow separation process.

The CliniMACS Plus CD14 System passes the antibody-labeled suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled CD14 positive target cells while the non-labeled cells (non-target cells) flow through the column. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged, and the magnetic field is removed. The target cells are collected in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, cellular starting product, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® CD14 Reagent

Intended purpose

- The CliniMACS® CD14 Reagent is intended for the *in vitro* enrichment of human CD14 positive cells from heterogeneous hematologic cell populations in combination with the CliniMACS System only.

- The CliniMACS Plus CD14 System is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS CD14 Reagent, the CliniMACS Tubing Set TS, and CliniMACS PBS/EDTA Buffer.

Composition

Each CliniMACS CD14 Reagent vial contains 7.5 mL of a colloidal solution of iron-dextran beads conjugated to murine monoclonal CD14 antibody in PBS/EDTA buffer stabilized with Poloxamer 188 (0.03% w/v).

Side-effects

- If the enriched CD14 positive cells are infused into patients, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndromes. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease could be more susceptible to iron-dextran reactions.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Precautions

- Before use in human applications, the suitability of target cells must be demonstrated regarding indication, quality and quantity.
- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively within the responsibility of the user.
- The enrichment of CD14 positive cells using the CliniMACS CD14 Reagent must be performed by trained operators only.
- Operators using the CliniMACS CD14 Reagent should have experience in the separation of cells from bone marrow or peripheral blood.
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- The CliniMACS CD14 Reagent is for *in vitro* use only. Do not inject or infuse the reagent directly into the patient. Not for parenteral application.
- The reagentis not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The reagent is for single use only (see fig. 4.1-1).
- Do not use the reagent after the use-by date printed on the vial label (see fig. 4.1-2).
- Do not use the reagent if package is damaged (see fig. 4.1-3). Use undamaged and sealed vials only.
- Store at +2 °C to +8 °C (see fig. 4.1-4). Do not freeze.

Further information

- The CliniMACS CD14 Reagent is tested for endotoxins.
- The reagent is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

Important

- This protocol requires the use of components which are not part of the CliniMACS Plus CD14 System (e.g., HSA). Therefore, all materials used must be pharmaceutical grade or FDA-licensed for human use.

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

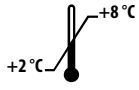


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Fig. 4.1-5: Sterilized using aseptic processing techniques.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

II. First actions required

CliniMACS® Materials required

- **CliniMACS® CD14 Reagent, REF 272-01**
  One vial of reagent required for use with the CliniMACS Plus CD14 System. If the labeling volume must be adjusted to 100 mL, a second CliniMACS CD14 Reagent vial must be used.

- **CliniMACS Tubing Set TS, REF 161-01**
  One CliniMACS Tubing Set TS must be used for the enrichment of CD14 positive cells.

- **CliniMACS PBS/EDTA Buffer, REF 700-25**
  CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. For the preparation procedure, one liter of buffer is required. For the separation, up to two liters of buffer are required (see table 4.1-1). Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v). Only FDA-licensed HSA should be used.

Note

The application capacity for the enrichment of CD14 positive cells using the CliniMACS Plus CD14 System is 4×10^9 CD14 positive cells out of a total cell number to 20×10^9. The optimal labeling volume for the magnetic labeling of CD14 positive cells must not exceed 50 g (±5 g). If the labeling volume exceeds 50 g, a second vial CliniMACS CD14 Reagent must be used.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Additional materials required

- **Cell Preparation Bag**
  One 600 mL transfer tag, suitable for centrifugation, as well as one sampling site coupler and one plasma transfer set, for use during the cell preparation procedure.

- **Plasma Waste Bag and Wash Waste Bag**
  Two 600 mL transfer bags, suitable for centrifugation.

- **Cell Collection Bag**
  One 150 mL or one 600 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set.

- **If the replacement of the Negative Fraction Bag and the Buffer Waste Bag is necessary (see table 4.1-1):**
  Two 1000 mL transfer bags in combination with two Luer/Spike Interconnector to connect the bags to the tubing set.

- **If more than one liter of buffer is required for the separation (see table 4.1-1):**
  One plasma transfer set to connect the buffer bags.

- Pre-system filter.

- Locking forceps.

- Appropriate syringes (1 mL, 10 mL) and hypodermic 20 gauge needles.

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).

- Sample tubes.

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3: "Equipment required".

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 "Additional materials required".

Important

After input of sample parameters (STEP 2), the CliniMACS Plus Software calculates the volumes that will be collected in the Priming Waste Bag, Cell Collection Bag, Negative Fraction Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volume of 500 mL, replacement of bags according to the following overview of materials required is necessary.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Overview of materials required

Cell preparation		
Total cells (WBCs)	20×10^9	20×10^9
Labeled cells	$\leq2\times10^9$	$2–4\times10^9$
Number of CliniMACS CD14 Reagent vials	1 or 2	1 or 2
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	1 liter
Plasma transfer set	1	1
CliniMACS Plus Separation		
Negative Fraction Bag	-	Replace original bags of the tubing set with 1000 mL transfer bags
Buffer Waste Bag	-	
Priming Waste Bag	-	-
Cell Collection Bag	150 mL	600 mL
Luer/Spike Interconnector	1	3
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	2 liters
Plasma transfer set	-	1

Table 4.1-1: Materials required for cell preparation and separation of CD14 positive cells.

Note

- The optimal labeling volume for the magnetic labeling of CD14 positive cells must not exceed 50 g (±5 g). If the labeling volume exceeds 50 g, a second vial CliniMACS CD14 Reagent must be used.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement each required liter of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v), i. e. add 5 g HSA per liter buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

A master copy of a worksheet is provided with the user manual to record important information and data generated during sample handling and preparation.

- Label one transfer bag (see table 4.1-1) as: **Cell Collection**. (This should include patient identification, date and time of run, and operator identification.) Insert a Luer/Spike Interconnector into the outside port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight on the worksheet (C_1).

- Label one 600 mL transfer bag as: **Cell Preparation**. (should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight on the worksheet (A_1).

- Label one 600 mL transfer bag as: **Plasma Waste**. (This should include patient identification, date, time of run and operator identification.)

- Label one 600 mL transfer bag as: **Wash Waste**. (This should include patient identification, date and time of run, operator identification.)

Important

- Note that **HSA is not a component of the CliniMACS Plus System**. Use only FDA-licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.

- Store the buffer for cell preparation at +19 °C to +25 °C. Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the tubing can vary during the preparation procedure, be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- All bag handling should be performed in a sterile environment (e.g., laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

III. Leukapheresis product

The following sections describe the recommended procedure for the preparation of the leukapheresis product using the Terumo Sterile Connection Device (TSCD).

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Analysis of leukapheresis product

Before starting the preparation of the leukapheresis product the following parameters should be determined.

- Total number of leukocytes,
- Percentage of CD14 positive cells,
- Total number of CD14 positive cells,
- Viability.

Recheck above mentioned parameters after the labeling procedure. This is necessary because the following three sample parameters must be entered in the software during the setup of the CliniMACS ^{plus} Instrument prior to loading the tubing set onto the instrument:

- Concentration of leukocytes per mL,
- Percentage of CD14 positive cells,
- Final volume of the cell sample (sample loading volume).

Other tests might be required depending on the intended use of the cells (e.g. phenotype of monocytes) or according to individual laboratory practice. Record all data on the worksheet.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Transfer of leukapheresis product into Cell Preparation Bag

1. Record the date and the start time on the worksheet before beginning to prepare the leukapheresis product.

2. Determine the volume of the original leukapheresis product by estimating 1 mL of leukapheresis product as equivalent to 1 g (1 g = 1 mL).

3. Holding the leukapheresis product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the Cell Preparation Bag to the original leukapheresis product bag.

5. Open the locking forceps to transfer the leukapheresis product material into the Cell Preparation. Use a tubing stripper to clear the tubing from any remaining blood. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation for further connections (see fig. 4.1-6). Retain the original leukapheresis bag until cell separation and final cell analysis have been completed.

7. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the leukapheresis product. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS PRODUCT (This should include patient identification.) and retain for cell analysis. Minimally, analysis of the WBC content, CD14 enumeration and viability testing are recommended.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight on the worksheet (A_2).

9. Determine the actual weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag (A_1) from the weight of the Cell Preparation Bag filled with leukapheresis product (A_2). Record the weight on the worksheet (A_3).

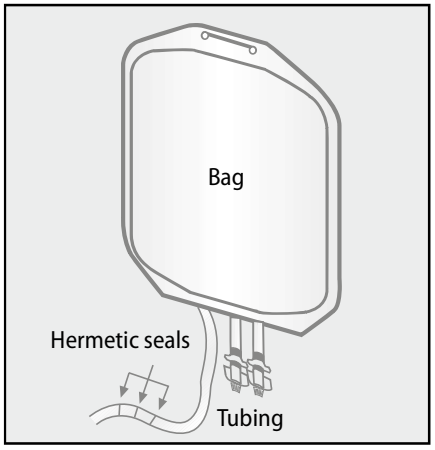


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

Worksheet equation:
$A_3 = A_2 - A_1$

Dilution of leukapheresis product

The leukapheresis product must be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Dilute the leukapheresis product with buffer to a total volume of 600 mL. Calculate the amount of buffer to be added using the following equation and record it on the worksheet (A_4). Do not use apheresis volumes higher than 200 g.

Worksheet equation: $A_4 = 600\ g - A_3$

Weight of buffer to be added (g) (A_4) = 600 g − Weight of leukapheresis product (g) (A_3)

1. Take a plasma transfer set and ensure the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer (A_4) to the Cell Preparation Bag.

5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer make three hermetic seals between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag on the worksheet (A_5).

Worksheet equation: $A_6 = A_5 - A_1$

9. Determine the total weight of diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag (A_1) from the weight of the filled Cell Preparation Bag (A_5). Record the weight on the worksheet (A_6).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Volume adjustment

1. For magnetic labeling of CD14 positive cells, the weight of the cell sample must not exceed 50 g (± 5 g). **If the labeling volume exceeds 50 g a second vial of CliniMACS CD14 Reagent must be used. When using two vials of reagent, the labeling volume must be adjusted to 100 g (+/– 5 g).**

   Calculate the amount of supernatant to be removed to adjust the sample to 50 g (or 100 g) using the equation below:

Weight of supernatant to be removed (g)	=	Weight of diluted leukapheresis product (g)	–	Optimal weight for labeling (g)

   Worksheet equation:
   $A_7 = A_6 - \text{optimal weight}$

   Record the weight of supernatant to be removed on the worksheet (A_7).

2. Place the empty Plasma Waste Bag on the balance and tare the balance.

3. Open the locking forceps next to the Cell Preparation Bag. Use the balance display to monitor the weight of the Plasma Waste Bag while removing the supernatant. Using the plasma extractor, carefully press out excess supernatant until the calculated "weight of supernatant to be removed" (A_7) is reached.

Important

- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is open before beginning the transfer. Release the extractor handle slowly.

- During removal of supernatant be careful not to lose cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

4. When the appropriate amount of supernatant has been transferred, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Record the weight of the supernatant removed on the worksheet (A_8).

5. Using the heat sealer, seal off the tubing to disconnect the Plasma Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag.

6. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

7. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight on the worksheet (A_9).

8. Determine the weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag (A_1) from the weight of the filled Cell Preparation Bag (A9). Record the calculated weight on the worksheet (A_{10}).

9. Keep the Plasma Waste Bag until the separation and final analysis of all cells has been accomplished.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

IV. Magnetic labeling of cells

One vial (7.5 mL) of CliniMACS CD14 Reagent is ready to use and sufficient for one preparation as described below. If the optimal labeling volume (50 g, +/– 5 g) for the magnetic labeling of CD14 positive cells is not achieved a second vial CliniMACS CD14 Reagent must be used. The reagent is not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze. The reagent is to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent are printed on the vial. DO NOT use the reagent after the use-by date.

Incubation with the CliniMACS® CD14 Reagent

1. Record the lot number (B_1) and use-by date of the CliniMACS® CD14 Reagent (B_2) on the worksheet.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from the CliniMACS CD14 Reagent vial (7.5 mL). A 10 mL syringe is sufficient to remove the contents of one reagent vial. A 20 mL syringe must be used to remove the contents of two vials of reagent. The syringe should be equipped with a 20 gauge needle.

3. **If the labeling volume of the leukapheresis product was adjusted to 100 g (±5 g), a second CliniMACS CD14 Reagent vial must be added.**

4. Using the injection port on the sampling site coupler, inject the entire volume of reagent into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 15 minute incubation.

5. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time on the worksheet (B_3).

6. Place the Cell Preparation Bag flat on the orbital rotator set to a speed of approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 15 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time on the worksheet (B_4).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Removal of excess reagent

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least one liter of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer (i.e., add 450 g to 550 g of buffer). To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of the filled Cell Preparation Bag on the worksheet (B_5). Close the locking forceps next to the Cell Preparation Bag.

5. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag on the balance. Tare the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

14. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much excess supernatant as possible from the Cell Preparation Bag. Record the amount of removed supernatant on the worksheet (B_6).

15 Close the locking forceps and using the heat sealer, seal off the tubing to disconnect the Wash Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.

16. Keep the Wash Waste Bag until the separation and final analysis of all cells has been accomplished.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Weigh the Cell Preparation Bag and record the weight on the worksheet (B_7). Determine the weight of the leukapheresis product after the wash by subtracting the weight of the empty Cell Preparation Bag (A_1) from the weight of the filled Cell Preparation Bag (B_7). Record the weight on the worksheet (B_8).

Worksheet equation:
$B_8 = B_7 - A_1$

19. Adjust sample loading volume: Calculate the amount of buffer necessary to adjust the weight of the cell suspension to approximately 100 g (B_9).

Worksheet equation:
$B_9 = 100\ g - B_8$

20. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

21. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

22. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

23. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

24. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

25. Resuspend the cell pellet in the Cell Preparation Bag. Avoid too intensive mixing of the cells. Ensure that all cells are resuspended.

Important

- If the supernatant removed is less than 500 mL a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
- For the preparation the volume of supernatant removed should be at least 500 mL.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

26. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (This should include patient identification.) and retain for cell analysis. WBC content, viability testing and CD14 enumeration are recommended.

Worksheet equation:
$B_{11} = B_{10} - A_1$

27. Weigh the Cell Preparation Bag and record the weight on the worksheet (B_{10}). Determine the weight of the leukapheresis product (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag (A_1) from the weight of the filled Cell Preparation Bag (B_{10}). Record the weight on the worksheet (B_{11}).

Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Worksheet for preparation and enrichment of CD14 positive cells

Patient Data _____

_____

Operator ID _____

Sample Data

Volume leukapheresis product: _____ mL

WBC concentration: _____ $\times 10^9$/L Total WBC: _____ $\times 10^9$

Percentage of monocytes: _____ %

Monocyte concentration: _____ $\times 10^9$/L Total monocytes: _____ $\times 10^9$

Transfer of leukapheresis product into Cell Preparation Bag

Starting: Date _____ Time _____ (A_1)

Remove leukapheresis product sample (0.5 mL) for cell count and flow cytometry (label **LEUKAPHERESIS PRODUCT).** Record analysis data in the table on page 7. Weight the empty and the filled bag.

Remove leukapheresis product sample ☐ (if yes ✓)

Weight of empty Cell Preparation Bag *(with locking forceps and sampling site coupler)* _____ g (A_1)

Weight of Cell Preparation Bag filled with leukapheresis product _____ g (A_2)

Weight of leukapheresis product (difference between filled and empty Cell Preparation Bag) $A_3 = A_2 - A_1$ _____ g (A_3)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Dilution of leukapheresis product

Dilute the leukapheresis product with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) to a total volume of 600 mL prior to magnetic labeling. Do not use apheresis volumes higher than 200 g.

Weight of buffer to be added
$A_4 = 600\ g - A_3$ _____ g (A_4)

Weight of filled Cell Preparation Bag
after dilution _____ g (A_5)

Weight of diluted leukapheresis product
$A_6 = A_5 - A_1$ _____ g (A_6)

Adjusting the leukapheresis product to 50 g

One vial of CliniMACS CD14 Reagent (7.5 mL) is capable of labeling up to 4×10^9 CD14 positive cells out of a total cell number of up to 20×10^9 cells. Optimal labeling requires a sample volume of 50 mL and one vial of CliniMACS CD14 Reagent. If the labeling volume exceeds 50 g, a second vial CliniMACS CD14 Reagent must be used.

Centrifugation conditions
Time:
g Force:
Temperature:

Use the equation below to calculate the weight of supernatant to be removed giving a final volume of 50 g.

Weight of supernatant to be removed (g) (A_7)	=	Weight of diluted leukapheresis product (A_6)	–	50 g

Weight of supernatant
to be removed
$A_7 = A_6 - 50\ g$ _____ g (A_7)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Remove the calculated weight of buffer to give a final volume of 50 g. Take care not to resuspend the cell pellet.

Actual weight of supernatant removed _____ g (A_8)

Weight of filled Cell Preparation Bag after volume adjustment _____ g (A_9)

Weight of leukapheresis product after volume adjustment
$A_{10} = A_9 - A_1$ _____ g (A_{10})

Magnetic labeling of CD14 positive cells

Lot number of CD14 Reagent _____ (B_1)

Use-by date of CD14 Reagent _____ (B_2)

Resuspend the cell pellet and add the entire content of one vial of CliniMACS CD14 Reagent. Mix carefully and incubate the Cell Preparation Bag for 15 min at room temperature on an orbital shaker at 25 rpm maximum.

Incubation

Incubation start time _____ (B_3)

Incubation stop time _____ (B_4)

Removal of excess reagent

Add buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) to a final volume of 600 mL for cell washing. Weight the filled Cell Preparation Bag prior to centrifugation and record the weight below.

Weight of the filled Cell Preparation Bag _____ g (B_5)

Centrifugation conditions

Time:

g Force:

Temperature:

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Remove as much supernatant as possible. Take care not to resuspend the pellet.

Weight of supernatant removed _____ g (B_6)

Weight of filled Cell Preparation Bag after washing procedure _____ g (B_7)

Weight of leukapheresis product after washing procedure
$B_8 = B_7 - A_1$ _____ g (B_8)

Final sample preparation

Adjust the volume of the leukapheresis product to 100 mL for loading on the CliniMACS plus Instrument.
Calculate the weight of buffer to be added using the equation below.
Remove leukapheresis product sample (0.5 mL) for cell count and flow cytometry analysis (label **ORIGINAL**).
Record all analysis data in table 1, page 7.

Weight of buffer to be added to reach 100 g
$B_9 = 100\ g - B_8$ _____ g (B_9)

Remove leukapheresis product sample (**ORIGINAL**) ☐ (if yes ✓)

Weight of filled Cell Preparation Bag after addition of buffer _____ g (B_{10})

Weight of leukapheresis product after addition of buffer (sample loading volume)
$B_{11} = B_{10} - A_1$ _____ g (B_{11})

CliniMACS® Separation (Run)

Date, time started run: Date _____ Time_____

Separation program **ENRICHMENT 1.1**

REF of tubing set used _____

Lot number of tubing set _____

Use-by date of tubing set _____

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Input of sample parameters

WBC concentration _____ × 10^6/mL

Percentage of labeled cells _____ %

Sample loading volume (mL) _____ mL

After selection of program and tubing set, enter WBC count, frequency of target cells and sample volume (calculation based on the results of the CD14 positive cells of the leukapheresis product).

The software calculates the required volumes of buffer and bags automatically. Prepare tubing set with buffer and bags as indicated with the calculation.

The empty bag weights of all fractions used for analysis and further processing must be recorded before starting the CliniMACS Run!

Load the Cell Preparation Bag onto the instrument, prime the system and press RUN to start the separation. Record the instrument process code after the run.

After the run has been completed: Calculation of the cells amounts

Target cell fraction of CD14 positive cells

(Cell Collection Bag)
Weight of empty Cell Collection Bag
(with locking forceps) _____ g (C_1)

Weight of filled Cell Collection Bag
after the run has been finished _____ g (C_2)

Weight of target cell fraction
$C_3 = C_2 - C_1$ _____ g (C_3)

Process code _____

Remove sample (0.5 mL) for cell count and flow cytometry analysis (label **target cell fraction**).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Non-target cell fraction

(Negative Fraction Bag)
Weight of empty
Negative Fraction Bag _____ g (C_4)

Weight of filled Negative Fraction Bag
after the run has been finished _____ g (C_5)

Weight of negative fraction
$C_6 = C_5 - C_4$ _____ g (C_6)

Remove sample (0.5 mL) for cell count and flow cytometry analysis (label **non-target cell fraction**).

Optional: Wash Fraction
(Buffer Waste Bag)
Weight of empty
Buffer Waste Bag _____ g (C_7)

Weight of filled Buffer Waste Bag
after the run has been finished _____ g (C_8)

Weight of buffer waste
$C_9 = C_8 - C_7$ _____ g (C_9)

Remove sample (0.5 mL) for cell count and flow cytometry analysis (label **wash fraction**).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Data analysis

This table summarizes data analysis required. Further analysis of additional parameters may be included based on the intended use of the target cells.

Fraction	Volume (mL)	Total cells ($\times 10^9$)	Percentage of CD14 positive cells (%)	Total viability (%)
Leukapheresis product				
ORIGINAL (after sample preparation, before separation)				
Target cells (Cell Collection Bag)				
Non-target cells (Negative Fraction Bag)				
Optional: Buffer Waste Bag (Wash fraction)				

Recovery of CD14 positive cells

$$= \frac{\text{Amount of CD14 positive cells in Cell Collection Bag}}{\text{Amount of CD14 positive cells in apheresis}} \times 100$$

$$= _____$$

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® Anti-Biotin Reagent

I. General information, warnings and precautions

General information

The CliniMACS® Plus Anti-Biotin System including the CliniMACS ^{plus} Instrument, the CliniMACS Anti-Biotin Reagent, the CliniMACS Tubing Set TS or CliniMACS Tubing Set LS, and CliniMACS PBS/EDTA Buffer is intended for the *in vitro* separation of human target cells prviously labeled with biotinylated antibodies or ligands.

The CliniMACS Anti-Biotin Reagent consists of murine monoclonal anti-biotin antibodies conjugated to super-paramagnetic particles.

The cells of interest are labeled with an appropriate biotinylated antibody or ligand, provided by the user. Subsequently, the cells are specifically labeled by incubation with the CliniMACS Anti-Biotin Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the separation in an automated continuous flow separation process.

It is possible to choose between an enrichment or a depletion strategy using the separation programs ENRICHMENT 1.1 or DEPLETION 2.1. These programs are designed for separation strategies of cells with medium to high antigen expression and a frequency of greater than 5% in the cell sample.

The CliniMACS Plus Anti-Biotin System passes the labeled suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled cells, while non-labeled cells flow through the column. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged, and magnetic field is removed. The target cells are collected in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, cellular starting product, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® Anti-Biotin Reagent

Intended purpose

- The CliniMACS® Anti-Biotin Reagent is intended for the *in vitro* enrichment or depletion of human cells previously labeled with biotinylated antibodies or ligands in combination with the CliniMACS Plus System only.

- The CliniMACS Plus Anti-Biotin System is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS Anti-Biotin Reagent, the CliniMACS Tubing Set TS, or the CliniMACS Tubing Set LS (depending on the separation program), and CliniMACS PBS/EDTA Buffer.

Composition

Each CliniMACS Anti-Biotin Reagent vial contains 7.5 mL of a colloidal solution of iron-dextran beads conjugated to murine monoclonal anti-biotin antibody in PBS/EDTA buffer stabilized with Poloxamer 188 (0.03% w/v).

Side-effects

- When the target cells are infused or injected into patients, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndromes. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptomes are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease appear to be highly susceptible to iron-dextran reactions.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Precautions

- Before use in human applications, the suitability of the target cells must be demonstrated regarding indication, quality and quantity.
- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively within the responsibility of the user.
- The separation of target cells using the CliniMACS Anti-Biotin Reagent must be performed by trained operators only.
- Operators using the CliniMACS Anti-Biotin Reagent should have experience in the separation of cells from bone marrow or peripheral blood.
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- The CliniMACS Anti-Biotin Reagent is for *in vitro* use only. Do not inject or infuse the reagent directly into the patient. Not for parenteral application.
- The reagentis not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The reagent is for single use only (see fig. 4.1-1).
- Do not use after the use-by date printed on the vial label (see fig. 4.1-2).
- Do not use the reagent if package is damaged (see fig. 4.1-3). Use undamaged and sealed vials only.
- Store at +2 °C to +8 °C (see fig. 4.1-4). Do not freeze.

Further information

- The CliniMACS Anti-Biotin Reagent is tested for endotoxins.
- The reagent is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

Important

This protocol requires the use of components which are not part of the CliniMACS Plus Anti-Biotin System (e.g., HSA). Therefore, all materials used must be pharmaceutical grade or FDA-licensed for human use.

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

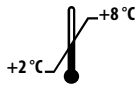


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Fig. 4.1-5: Sterilized using aseptic processing techniques.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

II. First actions required

Note

- The Flexible Labeling System using the CliniMACS Anti-Biotin Reagent provides the user with a number of selection and depletion strategies, therefore, the CliniMACS Materials must be adjusted to the specific needs of the separation strategy. An overview of the required separation specific materials is provided.
- The capacity for labeled cells depends on the characteristics of the cells and the primary antibody used. One vial of CliniMACS Anti-Biotin Reagent, 7.5 mL, is sufficient for processing up to 40×10^9 total cells. For up to 80×10^9 total cells, two vials of CliniMACS Anti-Biotin Reagent are needed.

CliniMACS® Materials required

- **CliniMACS® Anti-Biotin Reagent, REF 173-01**
  Using the CliniMACS Plus Anti-Biotin System, one vial is required for the normal scale application and two vials for the large scale application.

- **CliniMACS Tubing Set TS, REF 161-01**
  ENRICHMENT STRATEGY (ENRICHMENT 1.1):
  A CliniMACS Tubing Set TS must be used for the enrichment of cells.
  o r
  CliniMACS Tubing Set LS, REF 162-01
  DEPLETION STRATEGY (DEPLETION 2.1):
  A CliniMACS Tubing Set LS must be used for the depletion of cells.

- **CliniMACS PBS/EDTA Buffer, REF 700-25**
  CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. The amount of buffer varies, depending on the selected separation program and the number of labeled cells (see tables 4.1-1 and 4.1-2). Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 "Additional materials required".

Important

After input of sample parameters (STEP 2), the CliniMACS Software calculates the volumes that will be collected in the Priming Waste Bag, Cell Collection Bag, Negative Fraction Bag, and Buffer Waste Bag.If the volume of the calculated liquid exceeds the standard volume of 500 mL, replacement of bags according to the following overview of materials required is necessary.

Additional materials required

- **Cell Preparation Bag:**
  One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure.

- **Plasma Waste Bag and Wash Waste Bags:**
  Three or more 600 mL transfer bags, suitable for centrifugation.

- **Cell Collection Bag (see tables 4.1-1 and 4.1-2):**
  One 600 mL or one 1000 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set.

- **If the replacement of the Negative Fraction Bag, the Buffer Waste Bag, and/or the Priming Waste Bag is necessary (see tables 4.1-1 and 4.1-2):**
  Two to three 1000 mL transfer bags in combination with two to three Luer/Spike Interconnectors to connect the Negative Fraction and Buffer Waste bags to the tubing set. Replace the Priming Waste Bag using the TSCD (see "Equipment required").

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

- **If more than one liter of buffer is required for the separation (see tables 4.1-1 and 4.1-2):**
  One to two plasma transfer sets to connect the buffer bags.

- Pre-system filter.

- Locking forceps or slide clamps.

- Appropriate syringes (1 mL, 10 mL or 20 mL) and hypodermic 20 gauge needles.

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).

- Sample tubes.

- Individual biotinylated cell specific antibody or ligand.

- Optional: Human immunglobulin (IgG).

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3: "Equipment required".

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Overview of materials required

ENRICHMENT 1.1: Cell Peparation		
Total cells (WBC)	40×10^9	
No. of CliniMACS Anti-Biotin Reagent vials	1	
CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v))	2 liters	
Plasma transfer set	2	
ENRICHMENT 1.1: CliniMACS Plus Separation		
Labeled cells	$<2\times10^9$	$2–5\times10^9$
Negative Fraction Bag	-	Replace original bags of the tubing set by 1000 mL transfer bags
Buffer Waste Bag	-	
Priming Waste Bag	-	-
Cell Collection Bag	600 mL	600 mL
Luer/Spike Interconnector	1	3
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	2 liters
Plasma transfer set	-	1

Table 4.1-1: Materials required for the enrichment of cells (Separation program ENRICHMENT 1.1) using the CliniMACS Tubing Set TS.

DEPLETION 2.1: Cell Preparation		
Total cells (WBC)	40×10^9	80×10^9
No. of CliniMACS Anti-Biotin Reagent vials	1	2
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	2 liters	2 liters
Plasma transfer set	2	2

DEPLETION 2.1: CliniMACS Plus Separation			
Labeled cells	$<5\times10^9$	$5–13\times10^9$	$13–20\times10^9$
Negative Fraction Bag	-	Replace original bags of the tubing set by 1000 mL transfer bags	Replace original bags of the tubing set by 1000 mL transfer bags
Buffer Waste Bag	-		
Priming Waste Bag	-	-	
Cell Collection Bag	600 mL	1000 mL	1000 mL
Luer/Spike Interconnector	1	3	3
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	2 liters	3 liters
Plasma transfer set	-	1	2

Table 4.1-2: Materials required for the depletion of cells (Separation program DEPLETION 2.1) using the CliniMACS Tubing Set LS.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement each required liter of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v), i.e., add 5 g HSA per liter buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

- Label one transfer bag (see tables 4.1-1 and 4.1-2) as: **Cell Collection**. (This should include patient identification, date and time of run, and operator identification.) Insert a Luer/ Spike Interconnector into the port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL transfer bag as: **Cell Preparation**. (This should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL transfer bag as: **Plasma Waste.** (This should include patient identification, date, time of run and operator identification.)

- Label two 600 mL transfer bag as: **Wash Waste No. 1** and **Wash Waste No. 2**. (This should include patient identification, date and time of run, operator identification.)

Important

- Note that **HSA is not a component of the CliniMACS Plus System**. Use only FDA licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.

- Store the buffer for cell preparation at +19 °C to +25 °C. Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the coupler can vary during the preparation procedure be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

- In this chapter the cell preparation procedure for depletion strategies or enrichment strategies of cells with medium to high antigen expression and frequency higher than 5% is described.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- All bag handling should be performed in a sterile environment (e.g., laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

III. Cellular starting product

The following sections describe the recommended procedure for the preparation of the cellular starting product using the Terumo Sterile Connection Device (TSCD).

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Analysis of cellular starting product

Before starting the preparation of the cellular starting product the following parameters must be determined:

- Total number of leukocytes,
- Percentage of cells to be labeled,
- Total number of cells to be labeled,
- Viability.

Recheck the parameters after the labeling procedure. This is necessary because the following three sample parameters must be entered in the software during the setup of the CliniMACS ^{plus} Instrument prior to loading the tubing set onto the instrument.

- Concentration of leukocytes per mL,
- Percentage of cells to be labeled,
- Final volume of the cell sample (sample loading volume).

Other tests might be required depending on the intended use of the cells (e.g. T cell enumeration) or according to individual laboratory practice.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Transfer cellular starting product into Cell Preparation Bag

1. Record the date and the start time before beginning to prepare the cellular starting product.

2. Determine the volume of the original cellular starting product by estimating 1 mL of cellular starting product as equivalent to 1 g (1 g = 1 mL).

3. Holding the cellular starting product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the Cell Preparation Bag to the original cellular starting product bag.

5. Open the locking forceps to transfer the cellular starting product material into the Cell Preparation Bag. Use a tubing stripper to clear the tubing from any remaining blood. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see fig. 4.1-6). Retain the original leukapheresis bag until cell separation and analysis have been completed.

7. Disinfect the sampling site coupler surface. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the cellular starting product. Transfer the sample into a sample tube. Label the tube as CELLULAR STARTING PRODUCT (should include patient identification) and retain for cell analysis.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight.

9. Determine the actual weight of the cellular starting product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with cellular starting product. Record the weight.

Weight of leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

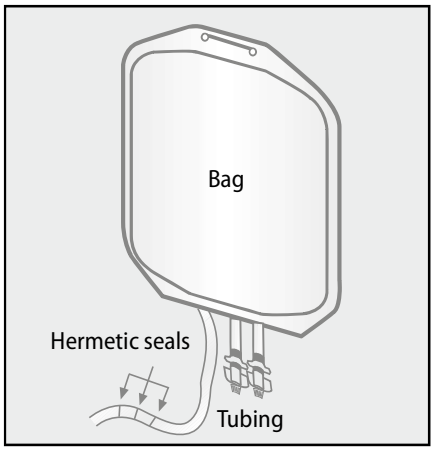


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Dilution of Cellular starting product

The cellular starting product must be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before labeling with the primary antibody or ligand. Calculate the amount of buffer to be added using the following equation and record it.

$$\text{Weight of buffer to be added (g)} = \text{Weight of cellular starting product (g)} \times 2$$

1. Take a plasma transfer set and ensure the slide clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the CliniMACS PBS/EDTA Buffer Bag.

2. Using the TSCD, connect the Buffer Bag tubing to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, make three hermetic seals between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

9. Determine the total weight of diluted cellular starting product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of diluted cellular starting product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

5. Remove the bags from the centrifuge, taking care not to resuspend the cell pellet. Load the Cell Preparation Bag onto the plasma extractor. Remove as much supernatant as necessary to give the sample the appropriate volume for primary antibody labeling. (Follow the instructions for use as provided by the manufacturer of the primary antibody.)

6. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag.

7. Keep the Plasma Waste Bag until the final separation and analysis of the all cells has been accomplished.

Important

- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is in open before beginning the transfer of supernatant. Release the extractor handle slowly.
- During removal of supernatant be careful not to lose cells.

Primary Antibody Labeling

1. Resuspend cell pellet and label cells with primary biotinylated antibody at time and titer recommended by the manufacturer of the antibody. Ensure that all cells are resuspended. (The primary antibody should be used at its optimal titer, i.e. with optimal staining intensity and no background staining. Follow the instructions for use as provided by the manufacturer of the primary antibody.)

2. Incubate the Cell Preparation Bag on an orbital rotator at 25 rpm. (Follow the instructions for use as provided by the manufacturer of the primary antibody.)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- For the normal scale application (one vial of CliniMACS Anti-Biotin Reagent), the volume of supernatant removed should be at least 500 mL.
- If the supernatant removed is less than 500 mL, two washing steps (instead of only one) are recommended. Otherwise, the removal of unbound reagent may be insufficient.

Removal of excess primary reagent: 1st Wash

Dilute the cellular starting product with CliniMACS PBS/EDTA Buffer supplemented with 0.5% HSA (w/v) to a total volume of 600 mL. Calculate the amount of buffer to be added using the following equation and record it.

Weight of buffer to be added (g)	=	600 mL	–	Weight of cellular starting product (g)

1. Dilute the cellular starting product following the steps described in the step “Dilution of the Cellular starting product“ (steps 1-8) to a total volume of 600 mL.
2. Determine the total weight of cellular starting product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of diluted cellular starting product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

3. Using the TSCD, connect the empty Wash Waste Bag No. 1 to the Cell Preparation Bag.
4. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.
5. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.
6. Centrifuge the cells at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).
7. Remove the bags from the centrifuge, taking care not to resuspend the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Volume Adjustment

1. For magnetic labeling of the cells, the optimal weight of the cell sample is a) 95 g (± 5 g), if one vial of CliniMACS Anti-Biotin Reagent is sufficient, or b) 190 g (± 5 g), if two reagent vials are needed (see tables 4.1-1 and 4.1-2).

   Calculate the amount of supernatant to be removed to adjust the sample to 95 g (or 190 g) using the equation below:

   a) Weight of supernatant to be removed (g) = Weight of diluted cellular starting product (g) – 95 g

   b) Weight of supernatant to be removed (g) = Weight of diluted cellular starting product (g) – 190 g

   Record the amount of supernatant to be removed.

2. Place the empty Plasma Waste Bag No. 1 on the balance and tare the balance.

3. Open the locking forceps next to the Cell Preparation Bag. Use the balance display to monitor the weight of the Wash Waste Bag while removing the supernatant. Using the plasma extractor, carefully press out excess supernatant until the calculated "weight of supernatant to be removed" is reached.

4. When the calculated weight of supernatant has been removed, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Record the weight of the supernatant removed.

5. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Wash Waste Bag No. 1.

6. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

7. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

8. Determine the weight of the cellular starting product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

   Weight of cellular starting product (g) = Weight after removing supernatant – Weight of empty Cell Preparation Bag (g)

9. Keep the Plasma Waste Bag until the separation and final analysis of all cells has been completed.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Note

In this chapter the cell preparation procedure for depletion strategies or separation strategies of cells with medium to high antigen expression and frequency higher than 5% is described.

IV. Magnetic Labeling of the Cells

The CliniMACS Anti-Biotin Reagent vials (7.5 mL each) are ready to use and sufficient for one application as described below. The reagents are not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze The reagent is to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent are printed on the reagent vial. DO NOT use the reagent after the use-by date.

Incubation with CliniMACS® Anti-Biotin Reagent

1. Record the lot number and use-by date of the CliniMACS® Anti-Biotin Reagent(s).

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from the CliniMACS Anti-Biotin Reagent vial(s) (7.5 mL). A 10 mL syringe is sufficient to remove the contents of one reagent vial or respectively, a 20 mL syringe is sufficient to remove the contents of two vials of reagent. The syringe used should be equipped with a 20 gauge needle.

3. Using the injection port on the sampling site coupler, inject the entire volume of reagent(s) into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

5. Place the Cell Preparation Bag flat on the orbital rotator, set to a speed of approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Removal of Excess Reagent: 2nd Wash

1. Insert the spike of a plasma transfer set to a port of a CliniMACS PBS/EDTA Buffer bag containing at least 500 mL of buffer (supplemented with 0.5% HSA). Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer. To stop the liquid flow, close the clamp on the plasma transfer set. Close the locking forceps next to the Cell Preparation Bag. Record the weight of the filled Cell Preparation Bag.

5. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (cellular starting product and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag No. 2 to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag No. 2 securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g for 15 min (without brake) at room emperature (+19 °C to +25 °C).

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- During removal of supernatant be careful not to lose cells.
- For the normal scale application (one vial of CliniMACS Anti-Biotin Reagent), the volume of the removed supernatant should be at least 500 mL.
- If the supernatant removed is less than 500 mL a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.

13. Place the Wash Waste Bag No. 2 on the balance. Tare the balance.

14. Open the locking forceps on the Cell Preparation Bag. Using the plasma extractor, remove as much supernatant from the Cell Preparation Bag as possible. Close the locking forceps next to the Cell Preparation Bag.

15. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Wash Waste Bag No. 2.

16. Keep the Wash Waste Bag No. 2 until the final separation and final analysis of all cells has been completed.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Weigh the Cell Preparation Bag and record the weight.

19. Determine the weight of the cellular starting product after the second wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of cellular starting product after the wash (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

20. Adjust sample loading volume: Calculate the weight of buffer necessary to adjust the weight of the cell suspension to approximately a) 100 g using 1 vial or b) 200 g using 2 vials.

a)	Weight of buffer to added (g)	=	100 g	–	Weight of cellular starting product after the wash (g)
b)	Weight of buffer to added (g)	=	200 g	–	Weight of cellular starting productafter the wash (g)

21. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

22. Place the Cell Preparation Bag on the balance and tare the balance.

23. Open the locking forceps next to the Cell Preparation Bag. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

24. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

25. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

26. Carefully mix bag content.

27. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (should include patient identification) and retain for cell analysis. WBC content, viability and the enumeration of Anti-Biotin positive cells are recommended.

28. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

29. Determine the weight of the cellular starting product after the addition of buffer (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag after buffer addition. Record the weight.

Weight of cellular starting product after buffer addition (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Proceed to STEP 2

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® CD56 Reagent

I. General information, warnings and precautions

General information

The CliniMACS® Plus CD56 System including the CliniMACS Plus Instrument, the CliniMACS CD56 Reagent, the CliniMACS Tubing Set TS, and CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human CD56 positive NK-cells from human peripheral blood cells.

The CD56 antigen is an isoform of the NCAM-family (neural cell adhesion molecule). On human haematopoetic cells CD56 is restricted to NK-cells and a subpopulation of T-lymphocytes. CD56 positive cells may be used for subsequent *in vitro* activation and expansions. Clinical application of NK-cells may include their use within a haploidentical PBSC transplantation or as effector cells in various hematological diseases.

The CliniMACS CD56 Reagent consists of selective CD56 monoclonal antibodies conjugated to super-paramagnetic particles. The CD56 positive cells are specifically labeled by incubation with the CliniMACS CD56 Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the separation in an automated continuous flow separation process.

The CliniMACS Plus CD56 System passes the antibody-labeled suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled CD56 positive cells (target cells) while the non-labeled cells (non-target cells) flow through the column. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged, and the magnetic field is removed. The target cells are collected in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, the cellular starting product, the CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® CD56 Reagent

Intended purpose

- The CliniMACS® CD56 Reagent is intended for the *in vitro* enrichment of human CD56 positive cells from heterogenous hematologic cell populations in combination with the CliniMACS System only.

- The CliniMACS Plus CD56 System is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS CD56 Reagent, the CliniMACS Tubing Set TS, and CliniMACS PBS/EDTA Buffer.

Composition

Each CliniMACS CD56 Reagent vial contains 7.5 mL of a colloidal solution of iron-dextran beads conjugated to murine monoclonal CD56 antibody in PBS/ EDTA buffer stabilized with Poloxamer 188 (0.03% w/v).

Side-effects

- When the enriched CD56 positive cells are infused into patients, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndromes. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease could be more susceptible to iron-dextran reactions.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Precautions

- Before use in human applications, the suitability of the target cells must be demonstrated regarding indication, quality and quantity.
- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively the responsibility of the user.
- The enrichment of CD56 positive cells using the CliniMACS CD56 Reagent must be performed by trained operators only.
- Operators using the CliniMACS CD56 Reagent should have experience in the separation of cells from bone marrow or peripheral blood.
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- The CliniMACS CD56 Reagent is for *in vitro* use only. Do not inject or infuse the reagent directly into the patient. Not for parenteral application.
- The reagent is not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The reagent is for single use only (see Fig. 4.1-1).
- Do not use the reagent after the use-by date printed on the vial label (see fig. 4.1-2).
- Do not use the reagent if package is damaged (see fig. 4.1-3). Use undamaged and sealed vials only.
- Store at +2 °C to +8 °C (see fig. 4.1-4). Do not freeze.

Further information

- The CliniMACS CD56 Reagent is tested for endotoxins.
- The reagent is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

Important

This protocol requires the use of components which are not part of the CliniMACS Plus CD56 System (e.g., HSA). Therefore, all materials used must be pharmaceutical grade or FDA-licensed for human use.

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

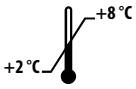


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Fig. 4.1-5: Sterilized using aseptic processing techniques.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

II. First actions required

Note

The application capacity for the enrichment of CD56 positive cells using the CliniMACS Plus CD56 System is to 5×10^9 CD56 positive cells out of a total cell number of 40×10^9 (WBC).

CliniMACS® Materials required

- **CliniMACS® CD56 Reagent, REF 701-46**
  One vial of reagent is required for use with the CliniMACS Plus CD56 System.

- **CliniMACS Tubing Set TS, REF 161-01**
  One CliniMACS Tubing Set TS must be used for the enrichment of CD56 positive cells.

- **CliniMACS PBS/EDTA Buffer, REF 700-25**
  CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. For the preparation procedure, one liter of buffer is required. For the separation, one to two liters of buffer are required. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 "Additional materials required".

Important

After input of sample parameters (STEP 2), the CliniMACS Software calculates the volumes that will be collected in the Priming Waste Bag, Cell Collection Bag, Negative Fraction Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volume of 500 mL, replacement of bags with 1000 mL transfer bags is necessary.

Additional materials required

- **Cell Preparation Bag:**
  One 600 mL transfer bag suitable for centrifugation as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure.

- **Plasma Waste Bag and Wash Waste Bag:**
  Two 600 mL transfer bags suitable for centrifugation.

- **Cell Collection Bag:**
  One 150 mL or one 600 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set.

- **If the replacement of the Negative Fraction Bag and/or the Buffer Waste Bag is necessary**
  Two 1000 mL transfer bags in combination with two Luer/Spike Interconnectors to connect the bags to the tubing set.

- **If more than one liter of buffer is required for the separation:**
  One plasma transfer set to connect the buffer bags.

- Pre-system filter.

- Locking forceps or slide clamps.

- Appropriate syringes (1 mL, 10 mL, 20 mL) and hypodermic 20 gauge needles.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).

- Sample tubes.

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3: "Equipment required".

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement each required liter of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5 % (w/v), i.e. add 5 g HSA per liter Buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

- One 600 mL transfer bag is already assembled and labeled as: **Cell Collection**. (Labeling should include patient identification, date and time of run, and operator identification.) Weigh the empty Cell Collection Bag and record the weight.

- Label one 600 mL transfer bag as: **Cell Preparation**. (This should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight on the worksheet.

- Label one 600 mL transfer bag as: **Plasma Waste**. (This should include patient identification, date, time of run and operator identification.)

- Label one 600 mL transfer bag as: **Wash Waste**. (This should include patient identification, date and time of run, operator identification.)

Important

- Note that **HSA is not a component of the CliniMACS Plus System**. Use only FDA licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.

- Store the buffer for cell preparation at +19 °C to +25 °C. Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the tubing can vary during the preparation procedure, be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- All bag handling should be performed in a sterile environment (e.g. laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

III. Leukapheresis product

The following sections describe the recommended procedure for preparing the leukapheresis product using the Terumo Sterile Connection Device (TSCD).

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Analysis of leukapheresis product

Before starting the preparation of the leukapheresis product the following parameters should be determined:

- Total number of leukocytes,
- Percentage of CD56 positive cells,
- Total number of CD56 positive cells,
- Viability.

Recheck above mentioned parameters after the labeling procedure. This is necessary because the following three sample parameters must be entered in the software during the setup of the CliniMACS ^{plus} Instument prior to loading the tubing set onto the instrument:

- Concentration of leukocytes per mL,
- Percentage of CD56 positive cells,
- Sample loading volume.

Other tests might be required depending on the intended use of the cells or according to individual laboratory practice.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Transfer Leukapheresis Product into Cell Preparation Bag

1. Record the date and the start time before beginning to prepare the leukapheresis product.

2. Determine the volume of the original leukapheresis product by estimating 1 mL of leukapheresis product as equivalent to 1 g (1 g = 1 mL).

3. Holding the leukapheresis product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the Cell Preparation Bag to the original leukapheresis product bag.

5. Open the clamp to transfer the leukapheresis product into the Cell Preparation Bag. Use a tubing stripper to clear the tubing from any remaining blood. Close the clamp next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see fig. 4.1-6). Retain the original leukapheresis bag until cell separation and analysis have been completed.

7. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the leukapheresis product. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS PRODUCT (should include patient identification) and retain for cell analysis. Minimally, analysis of the WBC content, target cell enumeration and viability are recommended.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the actual weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with leukapheresis product. Record the weight.

Weight of leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

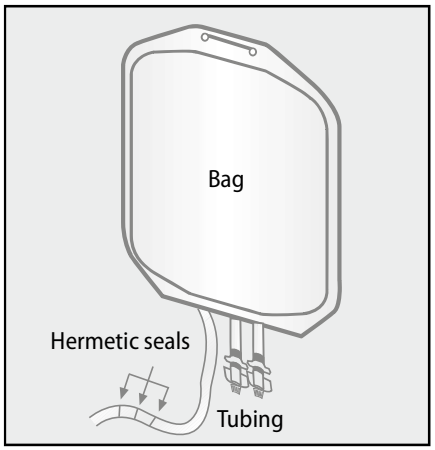


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Dilution of Leukapheresis Product

The leukapheresis product must be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Calculate the amount of buffer to be added using the following equation and record it.

Weight of buffer to be added (g)	=	600 g	–	Weight of leukapheresis product (g)

1. Take a plasma transfer set and ensure the slide clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the CliniMACS PBS/EDTA Buffer Bag.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

4. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, make three hermetic seals leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the weight of the diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of diluted leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is tared accurately.

5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Important

If the cell source has been previously processed (e.g. CD3 depletion) centrifuge the cells at 300×g for 15 min (without brake) at controlled room temperature (+19 °C to +25 °C).

Volume Adjustment

1. For magnetic labeling of CD56 positive cells, the weight of the cell sample must be adjusted to 95 g (± 5 g).

   Remove supernatant completely taking care not to resuspend the cell pellet during removal of supernatant. Close the clamp of the coupler, when the supernatant has been removed.When the supernatant has been removed, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag. Resuspend the cell pellet carefully.

   Adjust the weight of the Cell Preparation Bag by adding buffer. Calculate the weight of the Cell Preparation Bag filled with diluted leukapheresis product (LP) using the equation below:

Target weight of Cell Preparation Bag filled with diluted LP (g)	=	95 g	+	Weight of empty Cell Preparation Bag (g)

2. Insert the spike of a transfer set to a port of a buffer bag containing one liter of buffer. Confirm the clamp on the plasma transfer set is closed.

3. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

4. Tare the balance. Place the Cell Preparation Bag on the balance.

Important

- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is in the open position before beginning the transfer. Release the extractor handle slowly.
- During removal of supernatant be careful not to lose cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

5. Open the locking forceps next to Cell Preparation Bag. Then open the clamp on the plasma transfer set and fill the Cell Preparation Bag with buffer until the calculated "Target weight of Cell Preparation Bag filled with diluted leukapheresis product (g)" is reached. To stop the liquid flow, close the clamp on the plasma transfer set. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

8. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

9. Determine the weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

10. Keep the Plasma Waste Bag until the final separation and analysis of CD56 positive cells has been completed.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

IV. Magnetic Labeling of the Cells

One vial (7.5 mL) CliniMACS CD56 Reagent is ready to use and sufficient for one preparation as described below. The reagent is not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze. The reagent is to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent are printed on the vial. DO NOT use the reagent after the use-by date.

Incubation with CliniMACS® CD56 Reagent

1. Record the lot number and use-by date of the CliniMACS® CD56 Reagent.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from the CliniMACS CD56 Reagent vial (7.5 mL). A 10 mL syringe is sufficient to remove the contents of one reagent vial. The syringe should be equipped with a 20 gauge needle.

3. Using the injection port on the sampling site coupler, inject the entire volume of reagent into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

5. Place the Cell Preparation Bag flat on the orbital rotator set to a speed of approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Removal of Excess Reagent

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 500 mL of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer (i.e., add 500 g of buffer). To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of the filled Cell Preparation Bag.

5. Close the locking forceps next to the Cell Preparation Bag. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag on the balance. Tare the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

14. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much excess supernatant as possible from the Cell Preparation Bag.

15. Close the locking forceps and seal off the tubing to disconnect the Wash Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections

16. Keep the Wash Waste Bag until the final separation and analysis of CD56 positive cells has been accomplished.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag after the wash. Record the weight.

Weight of leukapheresis product after the wash (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

19. Adjust sample loading volume: Calculate the amount of buffer necessary to adjust the weight of the cell suspension to approximately 150 g.

Amount of buffer to be to be added (g)	=	150 g	–	Weight of leukapheresis product after the wash (g)

20. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

21. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

22. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

23. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next of the Cell Preparation Bag.

Important

- For the preparation, the volume of supernatant removed should be at least 500 mL.
- If the supernatant removed is less than 500 mL a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

24. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

25. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (should include patient identification) and retain for cell analysis.

26. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the addition of buffer (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of leukapheresis product after buffer addition (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® CD3 Reagent

I. General information, warnings and precautions

General information

The CliniMACS® Plus CD3 System including the CliniMACS plus Instrument, the CliniMACS CD3 Reagent, the CliniMACS Tubing Set LS or a CliniMACS Depletion Tubing Set, and CliniMACS PBS/EDTA Buffer is intended for the *in vitro* depletion of human CD3 positive cells from heterogeneous hematologic cell populations.

The CD3 antigen is present on mature human T cells, thymocytes, and a subset of NK cells. It is associated with the T cell receptor (TCR) and is responsible for the signal transduction of the TCR. The CD3 antigen is a complex of five invariable chains: γ, δ, ε, ζ and η. The CD3 antibody recognizes all T cells, i.e. it reacts with 70-80% of human peripheral blood lymphocytes and with 65-85% of thymocytes. The epitope recognized by the antibody is located on the ε-chain of the CD3 complex.

The CliniMACS Plus CD3 System uses selective CD3 monoclonal antibodies conjugated to super-paramagnetic particles. The CD3 positive cells are specifically labeled by incubation with the CliniMACS CD3 Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the depletion in an automated continuous flow separation process.

The CliniMACS Plus CD3 System passes the antibody-labeled cell suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled CD3 positive cells (non-target cells), while the unlabeled cells (target cells) flow through the column. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged, and the magnetic field is removed. The target cells are collected in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, cellular starting product, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® CD3 Reagent

Intended purpose

- The CliniMACS® CD3 Reagent is intended for the *in vitro* depletion of human CD3 positive cells from heterogeneous hematologic cell populations. in combination with the CliniMACS System only.

- The CliniMACS Plus CD3 System is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS CD3 Reagent, the CliniMACS Tubing Set LS or the CliniMACS Depletion Tubing Set, and CliniMACS PBS/EDTA Buffer.

Composition

Each CliniMACS CD3 Reagent vial contains 7.5 mL of a colloidal solution of iron-dextran beads conjugated to murine monoclonal CD3 antibody in PBS/EDTA Buffer stabilized with Poloxamer 188 (0.03% w/v).

Side-effects

- When the CD3 depleted cell fraction is infused into patients, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndromes. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease could be more susceptible to iron-dextran reactions.

Important

This protocol requires the use of components which are not part of the CliniMACS Plus CD3 System (e.g., HSA). Therefore, all materials used must be pharmaceutical grade or FDA-licensed for human use.

Precautions

- Before use in human applications, the suitability of the CD3-depleted target cell fraction must be demonstrated regarding indication, quality and quantity.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively within the responsibility of the user.
- The depletion of CD3 positive cells using the CliniMACS CD3 Reagent must be performed by trained operators only.
- Operators using the CliniMACS CD3 Reagent should have experience in the separation of cells from bone marrow or peripheral blood.
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- The CliniMACS CD3 Reagent is for *in vitro* use only. Do not inject or infuse the reagents directly into the patient. Not for parenteral application.
- The CD3 positive cell fraction (non-target cells) is not intended for therapeutic use and may not be reinfused into patients.
- The reagent is not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The reagent is for single use only (see fig. 4.1-1).
- Do not use the reagent after the use-by date printed on the vial label (see fig. 4.1-2).
- Do not use the reagent if package is damaged (see fig. 4.1-3). Use undamaged and sealed vials only.
- Store at +2 °C to +8 °C (see fig. 4.1-4). Do not freeze.

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

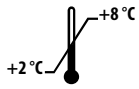


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Further information

- The CliniMACS CD3 Reagent is tested for endotoxins.
- The reagent is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

Fig. 4.1-5: Sterilized using aseptic processing techniques.

II. First actions required

CliniMACS® Materials required

- **CliniMACS® CD3 Reagent, REF 273-01**.
  Using the CliniMACS Plus CD3 System, one vial is required for the normal scale application and two vials for the large scale application.

- **CliniMACS Tubing Set LS, REF 162-01**
  o r
  CliniMACS Depletion Tubing Set, REF 261-01
  One CliniMACS Tubing Set LS or one CliniMACS Depletion Tubing Set must be used for the depletion of CD3 positive cells.

- **CliniMACS PBS/EDTA Buffer, REF 700-25.**
  The CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. For the preparation procedure, two liters buffer are required. For the separation, up to three liters buffer are required (see tables 4.1-1, 4.1-2, and 4.1-3). Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Additional materials required

- **Cell Preparation Bag**
  One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure.

- **Plasma Waste Bag and Wash Waste Bag**
  Two 600 mL transfer bags, suitable for centrifugation.

- **Cell Collection Bag (see tables 4.1-2 and 4.1-3)**:
  One to two 600 mL transfer bags in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set. If two Cell Collection Bags are required for the separation, use a plasma transfer set to connect both bags. One 600 mL transfer bag is already attached to the CliniMACS Depletion Tubing Set.

- **If the replacement of the Negative Fraction Bag, the Buffer Waste Bag, and/or the Priming Waste Bag is necessary (see tables 4.1-2 and 4.1-3):**
  Two to three 1000 mL transfer bags in combination with two Luer/Spike Interconnectors to connect the bags to the tubing set. Replace the Priming Waste Bag using the TSCD (see „Equipment required“).

Note

- **Using the CliniMACS Tubing Set LS**, the application capacity for the depletion of CD3 positive cells using the CliniMACS Plus CD3 System is 15×10^9 CD3 positive cells out of a total cell number not exceeding 40×10^9 (normal scale application using one vial of CliniMACS CD3 Reagent). For the depletion of up to 20×10^9 CD3 positive cells out of a total cell number of 80×10^9 (large scale application), two vials of CliniMACS CD3 Reagent are needed.

- **Using the CliniMACS Depletion Tubing Set**, the application capacity for the depletion of CD3 positive cells amounts to 15×10^9 CD3 positive cells out of a total cell number not exceeding 40×10^9 (normal scale application using one vial of CliniMACS CD3 Reagent). For the depletion of up to 30×10^9 CD3 positive cells out of a total cell number of 80×10^9 (large scale application), two vials of CliniMACS CD3 Reagent are needed.

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 “Additional materials required”.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

- **If more than one liter buffer is required for the separation (see tables 4.1-2 and 4.1-3):**
  One to two plasma transfer sets to connect the buffer bags.
- Pre-system filter.
- Locking forceps.
- Appropriate syringes (1 mL, 10 mL, 20 mL) and hypodermic 20 gauge needles.
- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).
- Pharaceutical grade immunoglobulin G.
- Sample tubes.

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3: "Equipment required".

Important

After input of sample parameters (STEP 2), the CliniMACS Plus Software calculates the volumes that will be collected in the Priming Waste Bag/Reapplication Bag, Cell Collection Bag, Negative Fraction Bag/Non-Target Cell Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volume of 500 mL, replacement of bags according to the following overview of materials required is necessary.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Overview of materials required

Cell preparation		
Total cells (WBC)	≤40×10^9 (normal scale)	≤80×10^9(large scale)
Labeled cells	≤15×10^9	15–30×10^9
Number of CliniMACS CD3 Reagent vials	1	2
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	2 liters	2 liters
Plasma transfer set	2	2
Sample loading volume	150 mL	250–300 mL

Table 4.1-1: Materials required for the cell preparation.

DEPLETION 2.1: CliniMACS Plus Separation with CliniMACS Tubing Set LS (REF 162-01)			
Labeled cells	≤5×10^9	5–13×10^9	13–20×10^9
Negative Fraction Bag	-	Replace the original bags of the tubing set by 1000 mL transfer bags.	Replace the original bags of the tubing set by 1000 mL transfer bags.
Buffer Waste Bag	-		
Priming Waste Bag	-	-	
Cell Collection Bag	600 mL	1–2 × 600 mL	2 × 600 mL
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	2 liters	3 liters
Luer/Spike Interconnector	1	3	3
Plasma transfer set	-	1-2	3

Table 4.1-2: Materials required for the depletion of CD3 positive cells using the CliniMACS Tubing Set LS.

DEPLETION 3.1: CliniMACS Plus Separation with CliniMACS Depletion Tubing Set (REF 261-01)		
Labeled cells	<29×10^9	≥29×10^9
Non-Target Cell Bag	No bag replacement necessary!	
Buffer Waste Bag		
Reapplication Bag		
Cell Collection Bag (one 600 mL bag is already attached to the CliniMACS Depletion Tubing Set)	-	1 × 600 mL (additionally)
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	
Luer/Spike Interconnector	-	-
Plasma transfer set	-	1

Table 4.1-3: Materials required for the depletion of CD3 positive cells using the CliniMACS Depletion Tubing Set.

Note

The materials required for the separation can also be determined by switching on the CliniMACS ^{plus} Instrument, selecting the separation program to be used (DEPLETION 2.1 for the CliniMACS Tubing Set LS and DEPLETION 3.1 for the CliniMACS Depletion Tubing Set) and entering the sample parameters. The CliniMACS Software will calculate the required sizes of bags to be connected to the tubing set and the required amount of buffer.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement each required liter of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5 % (w/v), i.e., add 5 g HSA per liter buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

- Label one (or two) transfer bag(s) (see tables 4.1-2 and 4.1-3) as: **Cell Collection**. One 600 mL transfer bag is already attached to the CliniMACS Depletion Tubing Set. (Labeling should include patient identification, date and time of run, and operator identification.) Insert a Luer/Spike Interconnector into the outside port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL transfer bag as: **Cell Preparation**. (This should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL Transfer Bag as: **Plasma Waste**. (This should include patient identification, date, time of run and operator identification.)

- Label one 600 mL Transfer Bag as: **Wash Waste**. (This should include patient identification, date and time of run, operator identification.)

Important

- Note that **HSA is not a component of the CliniMACS Plus System**. Use only FDA licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.

- Store the buffer for cell preparation at +19 °C to +25 °C. Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the coupler can vary during the preparation procedure be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- All bag handling should be performed in a sterile environment (e.g., laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

III. Leukapheresis product

The following sections describe the recommended procedure for the preparation of the leukapheresis product using the Terumo Sterile Connection Device (TSCD).

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Analysis of leukapheresis product

Before starting the preparation of the leukapheresis product the following parameters should be determined:

- Total number of leukocytes,
- Percentage of CD3 positive cells within leukocyte population,
- Total number of CD3 positive cells,
- Viability.

Determine the percentage/total number of all CD3-labeled cells (including unspecific CD3 binding, not only real T cells). Consider this number for the application specifications and for sample parameter input during the setup of the CliniMACS ^{plus} Instrument (see STEP 2 in the CliniMACS User Manual).

Other tests might be required depending on the intended use of the cells or individual laboratory practice.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Transfer leukapheresis product into Cell Preparation Bag

1. Record the date and the start time before beginning to prepare the leukapheresis product.

2. Determine the volume of the original leukapheresis product by estimating 1 mL of leukapheresis product as equivalent to 1 g (1 g ≙ 1 mL).

3. Holding the leukapheresis product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the Cell Preparation Bag to the original leukapheresis product bag.

5. Open the clamp to transfer the leukapheresis product material into the Cell Preparation Bag. Use a tubing stripper to clear the tubing of any remaining blood. Close the clamp next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see fig. 4.1-6). Retain the original leukapheresis bag until separation and final analysis have been completed.

7. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the leukapheresis product. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS PRODUCT (should include patient identification) and retain for cell analysis. Minimally, analysis of the WBC content, CD3 enumeration and viability testing are recommended.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the actual weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with leukapheresis product. Record the weight.

Weight of leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

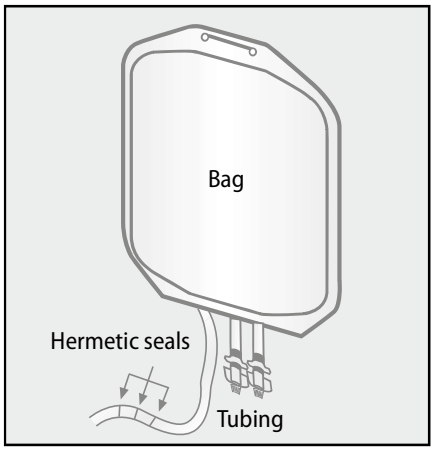


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Dilution of leukapheresis product

The leukapheresis product must be washed with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Dilute the leukapheresis product with buffer to a total volume of 600 mL. Calculate the amount of buffer to be added using the following equation and record it.

Weight of buffer to be added (g)	=	600 g	–	Weight of leukapheresis product (g)

1. Take a plasma transfer set and ensure the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

4. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, make three hermetic seals between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the weight of the diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of diluted leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Volume adjustment

1. For magnetic labeling of CD3 positive cells, the optimal weight of the cell sample must be adjusted to a) 95 g (± 5 g) for the normal scale application (one vial of CliniMACS CD3 Reagent) or b) 190 g (± 5 g) for the large scale application (two vials of CliniMACS CD3 Reagent), see table 4.1-4.

   Remove supernatant completely, taking care not to resuspend the cell pellet during removal of supernatant. When the supernatant has been removed, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag. Resuspend the pellet carefully.

   Adjust the weight of the Cell Preparation Bag by adding buffer. Calculate the target weight of the Cell Preparation Bag (CPB) filled with diluted leukapheresis product (LP) using the equation below, considering the volume of immunoglobulin to be added before labeling (see IV. Magnetic labeling of the cells):

   a) Target weight of CPB filled with diluted LP (g) = 95 g – IgG weight + Weight of empty CPB (g)

   b) Target weight of CPB filled with diluted LP (g) = 190 g – IgG weight + Weight of empty CPB (g)

Labeling	a) Normal scale application	b) Large scale application
Total cell number	≤40×10⁹ WBC	≤80×10⁹ WBC
Total CD3 positive cells	≤15×10⁹	15–30×10⁹
Number of CD3 Reagent vials	1	2
Optimal labeling weight	95 g ± 5g	190 g ± 5g
CliniMACS PBS/EDTA Buffer	2 liters	2 liters

Table 4.1-4: Reagents and buffer needed for labeling.

Important

- When using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is in the open position before beginning the transfer. Release the extractor handle slowly.

- During removal of supernatant be careful not to lose cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

2. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 1 liter of buffer. Confirm the clamp on the plasma transfer set is closed.

3. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

4. Tare the balance. Place the Cell Preparation Bag on the balance.

5. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and fill the Cell Preparation Bag with buffer until the calculated "Target weight of Cell Preparation Bag filled with diluted leukapheresis product" is reached. To stop the liquid flow, close the clamp on the plasma transfer set. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

8. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

9. Determine the weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

10. Keep the Plasma Waste Bag until the separation and final analysis of all cells have been completed.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

IV. Magnetic labeling of the cells

Incubation with clinical grade immunoglobulin

Disinfect the septum of the sampling site coupler of the Cell Preparation Bag. Use an appropriate sterile syringe and needle to add 1.5 mg human IgG per mL cellular product (e.g. 1.6 mL of Gamunex 10% for 95 mL cellular product). Mix the contents thoroughly. Incubate for 5 minutes at controlled room temperature by using a gentle rotating motion (e.g. 25 rpm on orbital rotator).

Incubation with the CliniMACS® CD3 Reagent

The CliniMACS® CD3 Reagent vials (7.5 mL each) are ready to use and sufficient for a preparation as described below. The reagents are not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze. The reagent is to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent are printed on the reagent vial. DO NOT use the reagent after the use-by date.

1. Record the lot number and use-by date of the CliniMACS CD3 Reagents.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from a) one or b) two CliniMACS CD3 Reagent vials (7.5 mL each). A 10 mL syringe is sufficient to remove the contents of one reagent vial for a normal scale application or, respectively, a 20 mL syringe is sufficient to remove the contents of two reagent vials for a large scale application. The syringe should be equipped with a 20 gauge needle.

3. Using the injection port on the sampling site coupler, inject the entire volume of reagent into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

5. Place the Cell Preparation Bag flat on the rotator, set to a speed of approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time.

Important

- Note that **human IgG is not a component of the CliniMACS Plus Plus System.** Use only pharmaceutical grade human IgG with appropriate approvals. Carefully read the package insert of the human IgG used; in particular the section regarding hypersensitivity reactions and the risk of infection that human IgG as a blood-derived product brings to all patients. All risks arising from these materials must be evaluated by the user.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Removal of excess reagent

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 500 mL of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer. To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of the filled Cell Preparation Bag.

5. Close the locking forceps next to the Cell Preparation Bag. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag on the balance. Tare the balance.

14. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much supernatant as possible from the Cell Preparation Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

15. Close the locking forceps and using the heat sealer, seal off the tubing to disconnect the Wash Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.

16. Keep the Wash Waste Bag until the separation and final analysis of all cells has been accomplished.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of leuka-pheresis product after the wash (g)	=	Weight of filled Cell Pre-paration Bag (g)	–	Weight of empty Cell Pre paration-Bag (g)

19. Adjust sample loading volume: Calculate the amount of buffer necessary to adjust the weight of the cell suspension to approximately a) 150 g (normal scale application) or b) 250–300 g (large scale application). For loading the labeled and washed cells on the tubing set, a maximum cell concentration of 0.4×10^9 cells per mL is recommended.

a)	Amount of buffer to be to be added (g)	=	150 g	–	Weight of leuka-pheresis product after the wash (g)
b)	Amount of buffer to be to be added (g)	=	250–300 g	–	Weight of leuka-pheresis product after the wash (g)

20. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

21. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

22. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

23. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

24. Using the heat sealer, seal off the tubing. Disconnect the buffer bag.

25. Resuspend the cell pellet in the Cell Preparation Bag. Avoid too intensive mixing of the cells. Ensure that all cells are resuspended.

26. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (should include patient identification) and retain for cell analysis. WBC content, CD3 enumeration and viability testing are recommended.

27. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the addition of buffer (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of leuka-pheresis product after buffer addition (g)	=	Weight of filled Cell Preparation-Bag (g)	–	Weight of empty Cell Preparation-Bag (g)

Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® CD3/56 Reagent Combination

I. General information

The CliniMACS® Plus CD3/CD56 System consists of a two-step process to generate NK cells. The process is used *in vitro* to first deplete CD3 positive cells and then enrich for CD56 positive cells from heterogeneous hematological cell populations; this cellular product may then be used in cases where purified NK cell treatment is clinically indicated.

The detailed procedures for the CliniMACS Plus CD3 System and the CliniMACS Plus CD56 System are provided as two separate chapters in the CliniMACS User Manual (US-Edition) that must be performed sequentially.

To obtain a purified NK cell product, the user should first refer to chapter 4, STEP 1: "CliniMACS CD3 Reagent" to deplete the CD3 positive cells from the cellular harvest. The CD3 depletion process involves two phases: magnetic cell labeling of the CD3 positive cells prior to depletion (phase I) and the automated cell depletion process (phase II). The target cell fraction, depleted of T cells, must then be enriched for CD56 positive cells.

To perform the CD56 enrichment procedure the user should refer to chapter 4, STEP 1: "CliniMACS CD56 Reagent" The CD56 enrichment process also involves two phases: magnetic cell labeling of the CD56 positive cells prior to enrichment (phase I) and the automated cell enrichment process (phase II). The purified NK cell product (target cell fraction), previously depleted of T cells, and then enriched for CD56 positive cells, may then be used immediately after analysis or cryopreserved for later infusion into the patient.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® CD19 Reagent

I. General information, warnings and precautions

General information

The CliniMACS® Plus CD19 System including the CliniMACS ^{plus} Instrument, the CliniMACS CD19 Reagent, the CliniMACS Tubing Set LS or a CliniMACS Depletion Tubing Set, and CliniMACS PBS/EDTA Buffer is intended for the *in vitro* depletion of human CD19 positive cells from heterogeneous hematologic cell populations.

The CD19 antigen is a critical signal transduction molecule that regulates B lymphocyte development, activation, and differentiation. As a B cell lineage marker, CD19 is expressed from the early pro-B cell stage to the B cell lymphoblast stage but the expression is downregulated upon B cell maturation to plasma cells. The CD19 antigen is further expressed on most malignant B cells and a subset of follicular dendritic cells.

The CliniMACS Plus CD19 System uses selective CD19 monoclonal antibodies conjugated to super-paramagnetic particles. The CD19 positive cells are specifically labeled by incubation with the CliniMACS CD19 Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the depletion in an automated continuous flow separation process.

The CliniMACS Plus CD19 System passes the antibody-labeled cell suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled CD19 positive cells (non-target cells) while the unlabeled cells (target cells) flow through the column. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged, and the magnetic field is removed. The target cells are collected in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, cellular starting product, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® CD19 Reagent

Intended purpose

- The CliniMACS® CD19 Reagent is intended for the *in vitro* depletion of human CD19 positive cells from heterogeneous hematologic cell populations in combination with the CliniMACS System only.

- The CliniMACS Plus CD19 System is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS CD19 Reagent, the CliniMACS Tubing Set LS, and CliniMACS PBS/EDTA Buffer.

Composition

Each CliniMACS CD19 Reagent vial contains 7.5 mL of a colloidal solution of iron-dextran beads conjugated to murine monoclonal CD19 antibody in PBS/EDTA buffer stabilized with Poloxamer 188 (0.03% w/v).

Side-effects

- When the CD19 depleted cell fraction is infused into patients, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndromes. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease could be more susceptible to iron-dextran reactions.

Important

This protocol requires the use of components which are not part of the CliniMACS Plus CD19 System (e.g., HSA). Therefore all materials used must be pharmaceutical grade or FDA-licensed for human use.

Precautions

- Before use in human applications, the suitability of the CD19-depleted target cell fraction must be demonstrated regarding indication, quality and quantity.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

- Further processing and use for clinical appplications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively within the responsibility of the user.
- The depletion of CD19 positive cells using the CliniMACS CD19 Reagent must be performed by trained operators only.
- Operators using the CliniMACS CD19 Reagent should have experience in the separation of cells from bone marrow or peripheral blood.
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- The CliniMACS CD19 Reagent is for *in vitro* use only. Do not inject or infuse the reagents directly into the patient. Not for parenteral application.
- The CD19 positive cell fraction (non-target cells) is not intended for therapeutic use and may not be reinfused into patients.
- The reagent is not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The reagent is for single use only (see fig. 4.1-1).
- Do not use the reagent after the use-by date printed on the vial label (see fig. 4.1-2).
- Do not use the reagent if package is damaged (see fig. 4.1-3). Use undamaged and sealed vials only.
- Store at +2 °C to +8 °C (see fig. 4.1-4). Do not freeze.

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

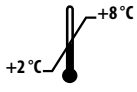


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Further information

- The CliniMACS CD19 Reagent is tested for endotoxins.
- The reagent is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

Fig. 4.1-5: Sterilized using aseptic processing techniques.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

II. First actions required

CliniMACS® Materials required

- **CliniMACS® CD19 Reagent, REF 273-01**.
  Using the CliniMACS Plus CD19 System, one vial of reagent is required for the normal scale application and two vials of reagent are required for the large scale application.

- **CliniMACS Tubing Set LS, REF 162-01**
  One CliniMACS Tubing Set LS must be used for the depletion of CD19 positive cells.

- **CliniMACS PBS/EDTA Buffer, REF 700-25**.
  The CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. For the preparation procedure, one liter buffer is required. For the separation, up to two liters of buffer are required (see tables 4.1-1). Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Note

The application capacity for the depletion of CD19 positive cells using the CliniMACS Plus CD19 System is 5×10^9 CD19 positive cells out of a total cell number not exceeding 40×10^9 (normal scale application using one vial of CliniMACS CD19 Reagent). For the depletion of up to 10×10^9 CD19 positive cells out of a total cell number of 80×10^9 (large scale application), two vials of CliniMACS CD19 Reagent are needed.

Additional materials required

- **Cell Preparation Bag:**
  One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and one plasma transfer sets, for use during the cell preparation procedure.

- **Plasma Waste Bag and Wash Waste Bag:**
  Two 600 mL transfer bags, suitable for centrifugation.

- **Cell Collection Bag (see table 4.1-1):**
  One 600 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set.

- **If the replacement of the Negative Fraction Bag, and the Buffer Waste Bag, is necessary (see table 4.1-1):**
  Two 1000 mL transfer bags in combination with two Luer/Spike Interconnectors to connect the bags to the tubing set.

- **If more than one liter buffer is required for the separation (see table 4.1-1):**
  One plasma transfer sets to connect the buffer bag.

- Pre-system filter.

- Locking forceps.

- Appropriate syringes (1 mL, 10 mL, 20 mL) and hypodermic 20 gauge needles

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 “Additional materials required”.

Important

After input of sample parameters (STEP 2), the CliniMACS Plus Software calculates the volumes that will be collected in the Priming Waste Bag, Cell Collection Bag, Negative Fraction Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volume of 500 mL, replacement of bags according to the following overview of materials required is necessary.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).

- Sample tubes.

Overview of materials required

Cell preparation		
Total cells (WBC)	≤40×10^9 (normal scale)	≤80×10^9(large scale)
Labeled cells	≤ 5×10^9	10×10^9
Number of CliniMACS CD19 Reagent vials	1	2
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	1 liter
Plasma transfer set	1	1
Sample loading volume	100 mL	200 mL

Cell separation		
Negative Fraction Bag	–	Replace the original bags of the tubing set by 1000 mL transfer bags
Buffer Waste Bag	–	
Priming Waste Bag	–	–
Cell Collection Bag	600 mL	600 mL
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liters	2 liters
Luer/Spike Interconnector	1	3
Plasma transfer set	–	1

Table 4.1-1: Materials required for the cell preparation and separation of CD19 positive cells

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3: "Equipment required".

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- Note that **HSA is not a component of the CliniMACS Plus System**. Use only FDA licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.

- Store the buffer for cell preparation at +19 °C to +25 °C. Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the coupler can vary during the preparation procedure be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement each required liter of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5 % (w/v), i.e., add 5 g HSA per liter buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

- Label one transfer bag (see tables 4.1-1) as: **Cell Collection**. (This should include patient identification, date and time of run, and operator identification.). Insert a Luer/Spike Interconnector into the outside port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL transfer bag as: **Cell Preparation**. (This should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL Transfer Bag as: **Plasma Waste**. (This should include patient identification, date, time of run and operator identification.)

- Label one 600 mL Transfer Bag as: **Wash Waste**. (This should include patient identification, date and time of run, operator identification.)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

III. Leukapheresis product

The following sections describe the recommended procedure for the preparation of the leukapheresis product using the Terumo Sterile Connection Device (TSCD).

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Analysis of leukapheresis product

Before starting the preparation of the leukapheresis product the following parameters should be determined:

- Total number of leukocytes,
- Percentage of CD19 positive cells,
- Total number of CD19 positive cells,
- Viability.

Recheck above mentioned parameters after the labeling procedure. This is necessary because the following three sample parameters must to be entered in the software during the setup of the CliniMACS ^{plus} Instument prior to loading the tubing set onto the instrument:

- Concentration of leukocytes per mL,
- Percentage of CD19 positive cells,
- Sample loading volume.

Other tests might be required depending on the intended use of the cells or according to individual laboratory practice. Record all data.

Important

- All bag handling should be performed in a sterile environment (e.g. laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

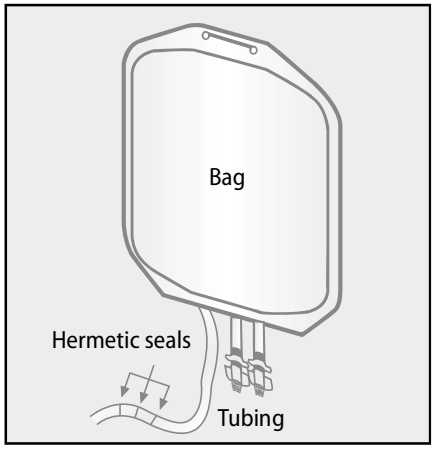


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

Transfer leukapheresis product into Cell Preparation Bag

1. Record the date and the start time before beginning to prepare the leukapheresis product.

2. Determine the volume of the original leukapheresis product by estimating 1 mL of leukapheresis product as equivalent to 1 g (1 g = 1 mL).

3. Holding the leukapheresis product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the Cell Preparation Bag to the original leukapheresis product bag.

5. Open the locking forceps to transfer the leukapheresis product material into the Cell Preparation Bag. Use a tubing stripper to clear the tubing of any remaining blood. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see fig. 4.1-6). Retain the original leukapheresis bag until separation and final analysis have been completed.

7. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the leukapheresis product. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS PRODUCT (should include patient identification) and retain for cell analysis. Minimally, analysis of the WBC content, CD19 enumeration, and viability testing are recommended.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the actual weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with leukapheresis product. Record the weight.

Weight of leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Pre paration Bag (g)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Dilution of leukapheresis product

The leukapheresis product must be washed with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Dilute the leukapheresis product with buffer to a total volume of 600 mL. Calculate the amount of buffer to be added using the following equation and record it.

Weight of buffer to be added (g)	=	600 g	–	Weight of leuka-pheresis product (g)

1. Take a plasma transfer set and ensure the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the weight of the diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of diluted leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Pre paration Bag (g)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Labeling	a) Normal scale application	b) Large scale application
Total cell number	≤40×10⁹ WBC	≤80×10⁹ WBC
Total CD19 positive cells	≤5×10⁹	5–10×10⁹
Number of CD19 Reagent vials	1	2
Optimal labeling weight	95 g ± 5g	190 g ± 5g
CliniMACS PBS/EDTA Buffer	2 liters	2 liters

Table 4.1-2: Reagents and buffer needed for labeling.

Important

- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is open before beginning the transfer. Release the extractor handle slowly. During removal of supernatant be careful not to lose cells.

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Volume adjustment

1. For magnetic labeling of CD19 positive cells, the optimal weight of the cell sample is a) 95 g (± 5 g) for the normal scale application (one vial CliniMACS CD19 Reagent) or b) 190 g (± 5 g) for the large scale application (two vials CliniMACS CD19 Reagent), see table 4.1-2.

   Remove supernatant completely, taking care not to resuspend the cell pellet during removal of supernatant. , When the supernatant has been removed, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag. Resuspend the pellet carefully.

   Place the Cell Preparation Bag with the cell pellet on the balance and tare it. Adjust the weight of the Cell Preparation Bag by adding buffer. Calculate the target weight of the Cell Preparation Bag (CPB) filled with diluted leukapheresisproduct (LP) using the equation below:

a) Target weight of Cell Preparation Bag filled with diluted LP (g) = 95 g + Weight of empty Cell Preparation Bag (g)

b) Target weight of Cell Preparation Bag filled with diluted LP (g) = 190 g + Weight of empty Cell Preparation Bag (g)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

2. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 1 liter of buffer. Confirm the clamp on the plasma transfer set is closed.

3. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

4. Tare the balance. Place the Cell Preparation Bag on the balance.

5. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and fill the Cell Preparation Bag with buffer until the optimal labeling volume, a) 95g b) 190g is reached. To stop the liquid flow, close the clamp on the plasma transfer set. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

8. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

9. Determine the weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

10. Keep the Plasma Waste Bag until the separation and final analysis of all cells has been completed.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

IV. Magnetic labeling of the cells

One vial (7.5mL) CliniMACS CD19 Reagent is ready to use and sufficient for one normal scale application as described below. For a large scale application, two vials of CliniMACS CD19 Reagent are required. The reagent is not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze. The reagent is to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent are printed on the reagent vial. DO NOT use the reagent after the use-by date.

Incubation with the CliniMACS® CD19 Reagent

1. Record the lot number and use-by date of the CliniMACS® CD19 Reagent (s).

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from a) one or b) two CliniMACS CD19 Reagent vials (7.5 mL each). A 10 mL syringe is sufficient to remove the contents of one vial for a normal scale application, or respectively, a 20 mL syringe is sufficient to remove the contents of two vials of reagent for a large scale application. The syringe should be equipped with a 20 gauge needle.

3. Using the injection port on the sampling site coupler, inject the entire volume of reagent into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

5. Place the Cell Preparation Bag flat on the orbital rotator at approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Removal of excess reagent

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 500 mL of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer. To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of the filled Cell Preparation Bag.

5. Close the locking forceps next to the Cell Preparation Bag. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag on the balance. Tare the balance.

14. Open the locking forceps next to the Cell Preparation Bag tubing. Using the plasma extractor, remove as much supernatant as possible from the Cell Preparation Bag. Close locking forceps next to the Cell Preparation Bag.

Important

If the supernatant removed is less than 500 mL, a total of two washing steps (instead of only one) is revommended. Otherwise the remval of unbound reagent may be insufficient.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

15. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Wash Waste Bag.

16. Keep the Wash Waste Bag until the separation and final analysis of all cells has been accomplished.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of leukapheresis product after the wash (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

19. Adjust sample loading volume: Calculate the amount of buffer necessary to adjust the weight of the cell suspension to approximately a) 100 g (normal scale application) or b) 200 g (large scale application). For loading the labeled and washed cells on the tubing set, a maximum cell concentration of 0.4×10^9 cells per mL is recommended.

a)	Amount of buffer to be to be added (g)	=	100 g	–	Weight of leukapheresis product after the wash (g)
b)	Amount of buffer to be to be added (g)	=	200 g	–	Weight of leukapheresis product after the wash (g)

20. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

21. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

22. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

23. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

24. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

25. Resuspend the cell pellet in the Cell Preparation Bag. Avoid too intensive mixing of the cells. Ensure that all cells are resuspended.

26. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (should include patient identification) and retain for cell analysis. WBC content, CD19 enumeration, and viability testing are recommended.

27. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the addition of buffer (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of leuka-pheresis product after buffer addition (g)	=	Weight of filled Cell Pre-paration Bag (g)	–	Weight of empty Cell Pre-paration Bag (g)

Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® CD3/CD19 Reagent Combination

I. General information, warnings, and precautions

General information

The CliniMACS® Plus CD3/CD19 System including the CliniMACS Plus Instrument, the CliniMACS CD3 Reagent and the CliniMACS CD19 Reagent, the CliniMACS Tubing Set LS or the CliniMACS Depletion Tubing Set, and CliniMACS PBS/EDTA Buffer is intended for the simultaneous *in vitro* depletion of human CD3 positive cells and human CD19 positive cells from heterogeneous hematologic cell populations.

The CliniMACS CD3/CD19 System consists of selective CD3 and CD19 monoclonal antibodies conjugated to super-paramagnetic particles. The CD3 and CD19 positive cells are specifically labeled by incubation with the CliniMACS CD3 Reagent and the CliniMACS CD19 Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the depletion in an automated continuous flow separation process.

The CliniMACS Plus CD3/CD19 System passes the antibody-labeled cell suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled CD3 and CD19 positive cells (non-target cells) while the unlabeled cells (target cells) flow through the column. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged, and the magnetic field is removed. The target cells are collected in the Cell Collection Bag.

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, cellular starting product, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Note

- **Using the CliniMACS Tubing Set LS,** the application capacity for the depletion of CD3 positive cells and CD19 positive cells using the CliniMACS Plus CD3/CD19 System amounts to 15×10^9 CD3 positive cells and 5×10^9 CD19 positive cells out of a total cell number not exceeding 40×10^9 (large scale application using one vial of CliniMACS CD3 Reagent and one vial of CliniMACS CD19 Reagent).

- **Using the CliniMACS Depletion Tubing Set,** the application capacity for the depletion of CD3 positive cells and CD19 positive cells amounts to 15×10^9 CD3 positive cells and 5×10^9 CD19 positive cells out of a total cell number not exceeding 40×10^9 (large scale application using one vial of CliniMACS CD3 Reagent and one vial of CliniMACS CD19 Reagent). For the depletion of up to 30×10^9 CD3 positive cells and 10×10^9 CD19 positive cells out of a total cell number of 80×10^9 (large scale application), two vials of CliniMACS CD3 Reagent and two vials of CliniMACS CD19 Reagent are needed.

- For high numbers of labeled cells it is recommended to use a CliniMACS Depletion Tubing Set, in order to save processing time. (e.g. 20×10^9 labeled cells will be processed within five hours using the CliniMACS Tubing Set LS versus 2 hours using a CliniMACS Depletion Tubing Set).

CliniMACS® CD3/CD19 Reagent combination

For the intended purpose, side-effects, precautions and warnings for the CliniMACS® CD3 Reagent and CliniMACS CD19 Reagent, see CliniMACS CD3 Reagent chapter and CliniMACS CD19 Reagent chapter, "General information, warnings and precautions".

II. First actions required

CliniMACS® Materials required

- **CliniMACS® CD3 Reagent, REF 273-01.**

- **CliniMACS CD19 Reagent, REF 179-01.**
  Using the CliniMACS Plus CD3/CD19 System, one vial of CliniMACS CD3 Reagent and one vial of CliniMACS CD19 Reagent are required for a normale scale preparation. For a large scale application, two vials of CliniMACS CD3 Reagent and two vials of CliniMACS CD19 Reagent are needed.

- **CliniMACS Tubing Set LS, REF 162-01.**
  o r
  CliniMACS Depletion Tubing Set, REF 261-01.
  One CliniMACS Tubing Set LS or one CliniMACS Depletion Tubing Set must be used for the simultaneous depletion of CD3 positive and CD19 positive cells.

- **CliniMACS PBS/EDTA Buffer, REF 700-25.**
  CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. For the preparation procedure, two liters buffer are required. For the separation, up to three liters of buffer are required (see tables 4.1-1 and 4.1-2). Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Additional materials required

- **Cell Preparation Bag:**
  One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure.

- **Plasma Waste Bag and Wash Waste Bag:**
  Two 600 mL transfer bags, suitable for centrifugation.

- **Cell Collection Bag (see tables 4.1-1 and 4.1-2)**:
  One to two 600 mL transfer bags in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set. If two Cell Collection Bags are required for the separation, use a plasma transfer set to connect both bags. One 600 mL transfer bag is already attached to the CliniMACS Depletion Tubing Set.

- **If the replacement of the Negative Fraction Bag, the Buffer Waste Bag, and the Priming Waste Bag is necessary (see tables 4.1-1 and 4.1-2):**
  Two to three 1000 mL transfer bags in combination with two Luer/Spike Interconnectors to connect the bags to the tubing set. Replace the Priming Waste Bag using the TSCD (see chapter 3 "Equipment required").

- **If more than one liter of buffer is required for the separation (see tables 4.1-1 and 4.1-2):**
  One to two plasma transfer sets to connect the buffer bags.

- Pre-system filter.

- Locking forceps or slide clamps.

- Appropriate syringes (1 mL, 10 mL, 20 mL, 50 mL) and hypodermic 20 gauge needles.

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).

- Clinical grade immunoglobulin G.

- Sample tubes.

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3: "Equipment required".

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 "Additional materials required".

Important

After input of sample parameters (STEP 2), the CliniMACS Software calculates the volumes that will be collected in the Priming Waste Bag/Reapplication Bag, Cell Collection Bag, Negative Fraction Bag/Non-Target Cell Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volumes bags need to be exchanged according to tables 4.1-1 and 4.1-2.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Overview of materials required

DEPLETION 2.1: Cell preparation			
Total cells (WBC)	≤40×10^9		
Labeled cells	≤15×10^9 CD3 positive cells and ≤5×10^9 CD19 positive cells		
Number of CliniMACS CD3 and CD19 Reagent vials	1× CD3 and 1× CD19		
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v)	2 liters		
Plasma transfer set	2		
Sample loading volume	150 mL		
DEPLETION 2.1: CliniMACS Plus Separation with CliniMACS Tubing Set LS (REF 162-01)			
Labeled cells	≤5×10^9	5–13×10^9	13–20×10^9
Negative Fraction Bag	-	Replace original bags of the tubing set by **1000 mL** transfer bags.	Replace original bags of the tubing set by **1000 mL** transfer bags.
Buffer Waste Bag	-		
Priming Waste Bag	-	-	
Cell Collection Bag(s)	1× 600 mL	1–2× 600 mL	2× 600 mL
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v)	1 liter	2 liters	3 liters
Luer/Spike Interconnector	1	3	3
Plasma transfer set	-	1-2	3

Table 4.1-1: Materials required for the cell preparation and simultaneous depletion of CD3 and CD19 positive cells using the CliniMACS Tubing Set LS.

DEPLETION 3.1: Cell preparation		
Total cells (WBC)	≤40×10^9	≤80×10^9
Labeled cells	≤15×10^9 CD3 positive cells and ≤5×10^9 CD19 positive cells	15–30×10^9 CD3 positive cells and 5–10×10^9 CD19 positive cells
Number of CliniMACS CD3 and CD19 Reagent vials	1× CD3 and 1× CD19	2× CD3 and 2× CD19
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v)	2 liters	2 liters
Plasma transfer set	2	2
Sample loading volume	150 mL	250-300 mL
DEPLETION 3.1: CliniMACS Plus Separation with CliniMACS Depletion Tubing Set (REF 261-01)		
Labeled cells	<29×10^9 positive cells	≥29×10^9 positive cells
Non-Target Cell, Buffer Waste Bag, Reapplication Bag	No bag replacement necessary!	
Cell Collection Bag (one 600 mL bag is already attached to the CliniMACS Depletion Tubing Set)	-	1× 600 mL (additionally)
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v)	1 liter	
Luer/Spike Interconnector	-	-
Plasma transfer set	-	1

Table 4.1-2: Materials required for the cell preparation and simultaneous depletion of CD3 and CD19 positive cells using the CliniMACS Depletion Tubing Set.

Note :

The materials required for the separation can also be determined by switching on the CliniMACS ^{plus} Instrument, selecting the separation program to be used (DEPLETION 2.1 for the CliniMACS Tubing Set LS and DEPLETION 3.1 for the CliniMACS Depletion Tubing Set) and entering the sample parameters. The CliniMACS Software will calculate the required sizes of bags to be connected to the tubing set and the required amount of buffer.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement each required liter of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5 % (w/v), i.e., add 5 g HSA per liter buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

- Label one (or two) transfer bag(s) as (see tables 4.1-1 and 4.1-2): **Cell Collection**. One 600 mL transfer bag is already attached to the CliniMACS Depletion Tubing Set. (Labeling should include patient identification, date and time of run, and operator identification.) Insert a Luer/Spike Interconnector into the outside port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with the locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL transfer bag as: **Cell Preparation**. (This should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with the locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL transfer bag as: **Plasma Waste**. (This should include patient identification, date, time of run, and operator identification.)

- Label one 600 mL transfer bag as: **Wash Waste**. (This should include patient identification, date and time of run, and operator identification.)

Important

- Note that **HSA is not a component of the CliniMACS System**. Use only FDA licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.

- Store the buffer for cell preparation at +19 °C to +25 °C. Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the coupler can vary during the preparation procedure be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- All bag handling should be performed in a sterile environment (e.g. laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

III. Leukapheresis product

The following sections describe the recommended procedure for the preparation of the leukapheresis product using the Terumo Sterile Connection Device (TSCD).

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Analysis of leukapheresis product

Before starting the preparation of the leukapheresis product the following parameters should be determined:

- total number of leukocytes,
- percentage of CD3 and CD19 positive cells within leukocyte population,
- total number of CD3 and CD19 positive cells,
- viability.

Determine the percentage/total number of **all** CD3- and CD19-labeled cells (including unspecific CD3 and CD19 binding, not only real T and B cells). Consider this number for the application specifications and for sample parameter input during the setup of the CliniMACS ^{plus} Instrument (see STEP 2).

Other tests might be required according to individual laboratory practices and depending on the intended use of the cells. Record all data.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Transfer leukapheresis product into Cell Preparation Bag

1. Record the date and the start time before beginning to prepare the leukapheresis product.

2. Determine the volume of the original leukapheresis product by estimating 1 mL of leukapheresis product as equivalent to 1 g (1 g = 1 mL).

3. Holding the leukapheresis product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the Cell Preparation Bag to the original leukapheresis product bag.

5. Open the clamp to transfer the leukapheresis product material into the Cell Preparation Bag. Use a tubing stripper to clear the tubing from any remaining blood. Close the clamp.

6. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see fig. 4.1-1). Retain the original leukapheresis bag until separation and final analysis have been completed.

7. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the leukapheresis product. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS PRODUCT (should include patient identification) and retain for cell analysis. Minimally, analysis of the WBC content, viability testing and CD3/CD19 enumeration is recommended.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the actual weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with leukapheresis product. Record the weight.

Weight of leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

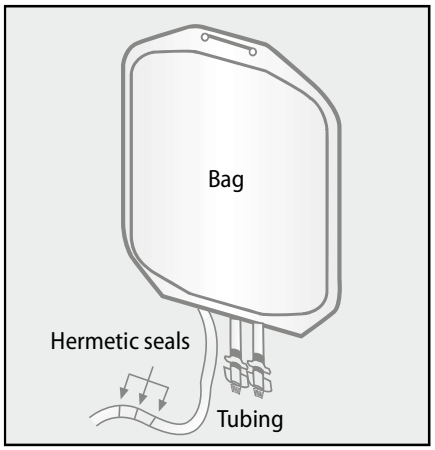


Fig. 4.1-1: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Dilution of leukapheresis product

The leukapheresis product must be washed with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Dilute the leukapheresis product with buffer to a total volume of 600 mL. Calculate the amount of buffer to be added using the following equation and record it.

Weight of buffer to be added (g)	=	600 g	–	Weight of leukapheresis product (g)

1. Take a plasma transfer set and ensure the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

4. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the total weight of the diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of diluted leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Volume adjustment

1. For magnetic labeling of CD19 and CD3 positive cells, the optimal weight of the cell sample is a) 87.5 g (± 5 g) (one vial CliniMACS CD3 Reagent and one vial CliniMACS CD19 Reagent) or b) 175 g (± 5 g) for the large scale application (two vials CliniMACS CD3 Reagent and two vials CliniMACS CD19 Reagent).

   Remove the supernatant completely, taking care not to resuspend the cell pellet during removal of supernatant. Close the clamp on the plasma transfer set, when the supernatant has been removed. Carefully resuspend the cell pellet. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag. Resuspend the pellet carefully.

   Adjust the weight of the Cell Preparation Bag by adding buffer. Calculate the target weight of the Cell Preparation Bag filled with diluted leukapheresis product (LP) using the equation below, considering the volume of immunoglobulin to be added before labeling (see IV. Magnetic labeling of the cells):

   a) Target weight of CPB filled with diluted LP (g) = 87.5 g – IgG weight + Weight of empty CPB (g)

   b) Target weight of CPB filled with diluted LP (g) = 175 g – IgG weight + Weight of empty CPB (g)

2. Insert the spike of a transfer set to a port of a buffer bag

Note

- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag are in the open position before beginning the transfer. Release the extractor handle slowly.
- During removal of supernatant be careful not to lose cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

containing at least 1 liter of buffer. Confirm the clamp on the plasma transfer set is closed.

3. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

4. Tare the balance. Place the Cell Preparation Bag on the balance.

5. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and fill the Cell Preparation Bag with buffer until the calculated "Target weight of Cell Preparation Bag filled with diluted leukapheresis product" is reached. To stop the liquid flow, close the clamp on the plasma transfer set. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

8. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

9. Determine the weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

10. Keep the Plasma Waste Bag until the separation and final analysis of all cells have been completed.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

IV. Magnetic labeling of the cells

Incubation with clinical grade immunoglobulin

Disinfect the septum of the sampling site coupler of the Cell Preparation Bag. Use an appropriate sterile syringe and needle to add 1.5 mg human IgG per mL cellular product (e.g. 1.6 mL of Gamunex 10% for 87.5 mL cellular product). Mix the contents thoroughly. Incubate for 5 minutes at controlled room temperature by using a gentle rotating motion (e.g. 25 rpm on orbital rotator).

Important

Note that **human IgG is not a component of the CliniMACS System**. Use only pharmaceutical grade human IgG approved in the country of use. Carefully read the package insert of the human IgG used; in particular the section regarding hypersensitivity reactions and the risk of infection that human IgG as a blood-derived product brings to all patients. All risks arising from these materials must be evaluated by the user.

Incubation with the CliniMACS® CD3 and the CliniMACS CD19 Reagent

The vials of CliniMACS® CD3 Reagent and CliniMACS CD19 Reagent (7.5 mL each) are ready to use and sufficient for a preparation as described below. The reagents are not for parenteral administration.

Store the reagents at +2 °C to +8 °C. DO NOT freeze. The reagents are to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagents are printed on the reagent vial. DO NOT use the reagents after the use-by date.

1. Record the lot number and use-by date of the CliniMACS Reagents.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from the CliniMACS Reagent vials (7.5 mL each). A 10 mL syringe is sufficient to take the contents of one vial for a normal scale application, or respectively, a 20 mL syringe is sufficient to remove the contents of two vials of reagent for a large scale application. The syringe should be equipped with a 20 gauge needle.

3. Using the injection port on the sampling site coupler, inject the entire volume of the reagents into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. After injecting the second reagent, immediately beginn the 30 min incubation time.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

5. Place the Cell Preparation Bag flat on the rotator at approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time.

Removal of excess reagent

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 1 liter of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer. To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of the filled Cell Preparation Bag.

5. Close the locking forceps next to the Cell Preparation Bag. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag on the balance. Tare the balance.

14. Open the locking forceps next to the Cell Preparation Bag tubing. Using the plasma extractor, remove as much excess supernatant as possible from the Cell Preparation Bag.

15. Close the locking forceps and seal off the tubing to disconnect the Wash Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.

16. Keep the Wash Waste Bag until the separation and final analysis of all cells has been accomplished.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of leukapheresis product after the wash (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

19. Adjust the sample loading volume: Calculate the amount of buffer necessary to adjust the weight of the cell suspension to approximately a) 150 g (large scale application) or b) 250–300 g (large scale application). For loading the labeled and washed cells on the tubing set, a maximum cell concentration of 0.4×10^9 cells per mL is recommended.

a)	Amount of buffer to be to be added (g)	=	150 g	–	Weight of leukapheresis product after the wash (g)
b)	Amount of buffer to be to be added (g)	=	250–300 g	–	Weight of leukapheresis product after the wash (g)

20. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

21. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

22. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

23. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

24. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

25. Resuspend the cell pellet in the Cell Preparation Bag. Avoid too intensive mixing of the cells. Ensure that all cells are resuspended.

26. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (should include patient identification) and retain for cell analysis Minimally, analysis of the WBC content, viability testing and CD3/CD19 enumeration is recommended.

27. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the addition of buffer (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of leukapheresis product after buffer addition (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® CD8 Reagent

I. General information, warnings and precautions

General information

The CliniMACS® Plus CD8 System including the CliniMACS ^{plus} Instrument, the CliniMACS CD8 Reagent, the CliniMACS Tubing Set LS or a CliniMACS Depletion Tubing Set, and CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment or depletion of human CD8 positive cells from heterogeneous hematologic cell populations.

The CD8 antigen is present on 20-30% of human peripheral blood lymphocytes. It is a disulfide-linked dimer, which exists either as a CD8 α homodimer or as a CD8 α/β heterodimer. CD8 is strongly expressed on CD8 positive T cells and thymocytes and dimly on a subset of NK cells and CD8 positive γ/δ T cells. CD8 acts as a co-receptor with MHC class I-restricted T cell receptors in antigen recognition.

The CliniMACS Plus CD8 System uses selective CD8 monoclonal antibodies conjugated to super-paramagnetic particles. The CD8 positive cells are specifically labeled by incubation with the CliniMACS CD8 Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the separation in an automated continuous flow separation process.

For CD8 positive cells either an enrichment or a depletion strategy can be chosen. For the enrichment of CD8 positive cells, the separation program ENRICHMENT 1.1 must be used. The separation program DEPLETION 2.1 must be chosen for the depletion of CD8 positive cells.

The CliniMACS Plus CD8 System passes antibody-labeled suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled CD8 positive cells (non-target cells), while the unlabeled cells (target cells) flow through the column. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged, and the magnetic field is removed. The target cells are collected in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, cellular starting product, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® CD8 Reagent

Intended purpose

- The CliniMACS® CD8 Reagent is intended for the *in vitro* enrichment or depletion of human CD8 positive cells from heterogeneous hematologic cell populations.

- The CliniMACS Plus CD8 System is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS CD8 Reagent, the CliniMACS Tubing Set TS or a CliniMACS Tubing Set LS, and CliniMACS PBS/EDTA Buffer.

Composition

Each CliniMACS CD8 Reagent vial contains 7.5 mL of a colloidal solution of iron-dextran beads conjugated to murine monoclonal CD8 antibody in PBS/EDTA buffer stabilized with Poloxamer 188 (0.03% w/v).

Side-effects

- When the CD8 enriched or depleted cell fraction is infused or injected into patients, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndroms. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease could be more susceptible to iron-dextran reactions.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Precautions

- Before use in human applications, the suitability of the target cells must be demonstrated regarding indication, quality and quantity.
- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively within the responsibility of the user.
- The separation of CD8 positive cells using the CliniMACS CD8 Reagent must be performed by trained operators only.
- Operators using the CliniMACS CD8 Reagent should have experience in the separation of cells from bone marrow or peripheral blood.
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- For *in vitro* use only. Do not inject or infuse the reagent directly into the patient. Not for parenteral application.
- The CliniMACS CD8 Reagent is not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The CliniMACS CD8 Reagent is for single use only (see fig. 4.1-1).
- Do not use the reagent after the use-by date printed on the vial label (see fig. 4.1-2).
- Do not use the reagent if package is damaged (see fig. 4.1-3). Use undamaged and sealed vials only.
- Store at +2 °C to +8 °C (see fig. 4.1-4). Do not freeze.

Further information

- The CliniMACS CD8 Reagent is tested for endotoxins.
- The reagent is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

Important

This protocol requires the use of components which are not part of the CliniMACS CD8 System (e.g., HSA). Therefore, all materials used must be pharmacuetical grade or FDA-licensed for human use.

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

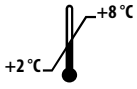


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Fig. 4.1-5: Sterilized using aseptic processing techniques.

II. First actions required

CliniMACS® Materials required

- **CliniMACS® CD8 Reagent, REF 275-01.**

Enrichment Strategy (ENRICHMENT 1.1):

- **CliniMACS Tubing Set TS, REF 161-01**
  A CliniMACS Tubing Set TS must be used for the normal scale preparation of CD8 positve cells.
  o r
  CliniMACS Tubing Set LS, REF 162-01.
  A CliniMACS Tubing Set LS must be used for a large scale preparation of CD8 positve cells.

Depletion Strategy (DEPLETION 2.1):
CliniMACS Tubing Set LS, REF 162-01.
A CliniMACS Tubing Set LS must be used for the normal scale and large scale preparation of CD8 positve cells.

- **CliniMACS PBS/EDTA Buffer, REF 700-25.**
  The CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. The amount of buffer needed for both steps varies depending on the selected separation program and the number of labeled cells (see table 4.1-2 and 4.1-1). Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Note

- The application capacity for the enrichment of CD8 positive cells using the CliniMACS Plus CD8 System amounts to 4×10^9 CD8 positive cells out of a total cell number not exceeding 40×10^9 (normal scale preparation) using one vial of CliniMACS CD8 Reagent. For the enrichment of up to 8×10^9 CD8 positive cells out of a total cell number of 80×10^9 (large scale preparation), two vials of CliniMACS CD8 Reagent are needed.

- The application capacity for the depletion of CD8 positive cells amounts to 4×10^9 CD8 positive cells out of a total cell number not exceeding 40×10^9 (normal scale preparation) using one vial of CliniMACS CD8 Reagent. For the depletion of up to 8×10^9 CD8 positive cells out of a total cell number of 80×10^9 (large scale preparation), two vials of CliniMACS CD8 Reagent are needed.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Additional materials required

- **Cell Preparation Bag:**
  One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure.

- **Plasma Waste Bag and Wash Waste Bag:**
  Two 600 mL transfer bags, suitable for centrifugation.

- **Cell Collection Bag (see tables 4.1-1 and 4.1-2):**
  One 600 mL transfer bags in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set. If two Cell Collection Bags are required for the separation, use a plasma transfer set to connect both bags.

- **If the replacement of the Negative Fraction Bag and/or the Buffer Waste Bag is necessary (see tables 4.1-1 and 4.1-2):**
  Two 1000 mL transfer bags in combination with two Luer/Spike Interconnectors to connect the bags to the tubing set.

- **If more than one liter buffer is required for the separation:**
  One to two plasma transfer sets to connect the buffer bags.

- Pre-system filter.

- Locking forceps.

- Appropriate syringes (1 mL, 10 mL, 20 mL or 50 mL) and hypodermic 20 gauge needles.

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).

- Sample tubes.

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3 "Equipment required".

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 "Additional materials required".

Important

After input of sample parameters (STEP 2), the CliniMACS Software calculates the volumes that will be collected in the Priming Waste Bag, Cell Collection Bag, Negative Fraction Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volume of 500 mL, replacement of bags according to table 4.1-1 and 4.1-2 is necessary.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Overview of materials required

ENRICHMENT 1.1: Cell preparation for the enrichment of CD8 positive cells		
Total cells (WBC)	40×10^9	$40–80\times10^9$
Labeled cells	$<4\times10^9$	$4–8\times10^9$
Number of CliniMACS CD8 Reagent vials	1	2
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	1 liter
Plasma transfer set	1	1
Sample loading volume	100 mL	200 mL
ENRICHMENT 1.1: Enrichment using the CliniMACS Tubing Set TS (REF 161-01)		
Labeled cells	$<2\times10^9$	$2\text{-}5\times10^9$
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	2 liters
Negative Fraction Bag	–	Replace original bags of the tubing set by **1000 mL** transfer bags
Buffer Waste Bag		
Priming Waste Bag		–
Cell Collection Bag	600 mL	600 mL
Luer/Spike Interconnector	1	3
Plasma transfer set	–	1

Table 4.1-1: Materials required for the enrichment of CD8 positive cells using separation program ENRICHMENT 1.1.

DEPLETION 2.1: Cell preparation for depletion of CD8 positive cells		
Total cells (WBC)	40×10^9	80×10^9
Labeled cells	$<4\times10^9$	$<8\times10^9$
No. of CliniMACS CD8 Reagent vials	1	2
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	1 liter
Plasma transfer set	1	1
DEPLETION 2.1: Depletion using the CliniMACS Tubing Set LS (REF 162-01)		
Labeled cells	$<5\times10^9$	$5–8\times10^9$
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	2 liters
Negative Fraction Bag	No bag replacement necessary	
Buffer Waste Bag		
Priming Waste Bag		
Cell Collection Bag	600 mL	600 mL
Luer/Spike Interconnector	1	1
Plasma transfer set	–	1

Table 4.1-2: Materials required for depletion of CD8 positive cells using separation program DEPLETION 2.1.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement each required liter of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5 % (w/v), i.e., add 5 g HSA per liter buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

- Label one transfer bag as: **Cell Collection**. (Labeling should include patient identification, date and time of run, and operator identification.) Insert a Luer/Spike Interconnector into the outside port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL transfer bag as: **Cell Preparation**. (This should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL Transfer Bag as: **Plasma Waste**. (This should include patient identification, date, time of run and operator identification.)

- Label one 600 mL Transfer Bag as: **Wash Waste**. (This should include patient identification, date and time of run, operator identification.)

Important

- Note that **HSA is not a component of the CliniMACS System**. Use only FDA licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.

- Store the buffer for cell preparation at +19 °C to +25 °C. Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the coupler can vary during the preparation procedure be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- All bag handling should be performed in a sterile environment (e.g. laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

III. Leukapheresis product

The following sections describe the recommended procedure for the preparation of the leukapheresis product using the Terumo Sterile Connection Device (TSCD).

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Analysis of leukapheresis product

Before starting the preparation of the leukapheresis product the following parameters should be determined:

- Volume and cell concentration to determine the total number of leukocytes,
- Percentage of CD8 positive cells within leukocyte population,
- Total number of CD8 positive cells,
- Viability.

Recheck these parameters after the labeling procedure. This is necessary because the following three sample parameters must be entered in the software during the setup of the CliniMACS ^{plus} Instument prior to loading the tubing set onto the instrument:

- Concentration of leukocytes per mL,
- Percentage of CD8 positive cells,
- Sample loading volume.

Other tests might be required depending on the intended use of the cells or individual laboratory practice.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Transfer leukapheresis product into Cell Preparation Bag

1. Record the date and the start time before beginning to prepare the leukapheresis product.

2. Determine the volume of the original leukapheresis product by estimating 1 mL of leukapheresis product as equivalent to 1 g (1 g = 1 mL).

3. Holding the leukapheresis product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the Cell Preparation Bag to the original leukapheresis product bag.

5. Open the locking forceps to transfer the leukapheresis product material into the Cell Preparation Bag. Use a tubing stripper to clear the tubing of any remaining blood. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see fig. 4.1-6). Retain the original leukapheresis bag until separation and final analysis have been completed.

7. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the leukapheresis product. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS PRODUCT (should include patient identification) and retain for cell analysis. Minimally, analysis of the WBC content, CD8 enumeration and viability testing are recommended.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the actual weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with leukapheresis product. Record the weight.

Weight of leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

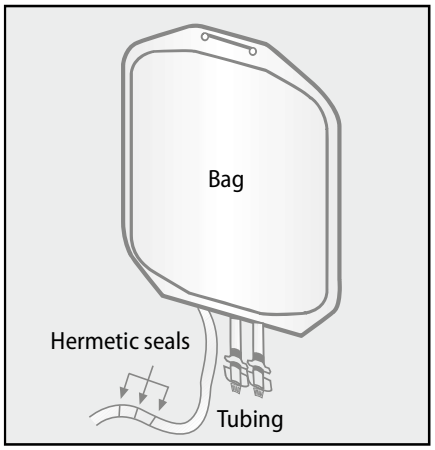


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Dilution of leukapheresis product

The leukapheresis product must be washed with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Calculate the amount of buffer to be added using the following equation and record it.

Weight of buffer to be added (g)	=	Weight of leukapheresis product (g)	×	2

1. Take a plasma transfer set and ensure the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

4. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the weight of the diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of diluted leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Volume adjustment

1. For magnetic labeling of CD8 positive cells, the optimal weight of the cell sample must be adjusted to a) 95 g (± 5 g) for the normal scale preparation (one vial of CliniMACS CD8 Reagent) or b) 190 g (± 5 g) for the large scale preparation (two vials of CliniMACS CD8 Reagent), see table 4.1-4.

   Remove supernatant completely, taking care not to resuspend the cell pellet during removal of supernatant. Close the locking forceps next to the Cell Preparation Bag, when the supernatant has been removed. Using the TSCD, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag. Resuspend the cell pellet carefully.

   Adjust the weight of the Cell Preparation Bag by adding buffer. Calculate the target weight of the Cell Preparation Bag (CPB) filled with diluted leukapheresis product (LP) using the equation below:

   a) Target weight of CPB filled with diluted LP (g) = 95 g + Weight of empty CPB (g)

   b) Target weight of CPB filled with diluted LP (g) = 190 g + Weight of empty CPB (g)

2. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 1 liter of buffer. Confirm the clamp on the plasma transfer set is closed.

Labeling	a) Normal scale preparation	b) Large scale preparation
Total cell number	≤40×10^9 WBC	≤80×10^9 WBC
Total CD8 positive cells	≤5×10^9	5–8×10^9
Number of CD8 Reagent vials	1	2
Optimal labeling weight	95 g ± 5g	190 g ± 5g
CliniMACS PBS/EDTA Buffer	2 liters	2 liters

Table 4.1-4: Reagents and buffer needed for labeling.

Important

- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is in the open position before beginning the transfer. Release the extractor handle slowly.

- During removal of supernatant be careful not to lose cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

3. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

4. Tare the balance. Place the Cell Preparation Bag on the balance.

5. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and fill the Cell Preparation Bag with buffer until the calculated "Target weight of Cell Preparation Bag filled with diluted leukapheresis product" is reached. To stop the liquid flow, close the clamp on the plasma transfer set. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

8. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

9. Determine the weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

10. Keep the Plasma Waste Bag until the separation and final analysis of all cells has been completed.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

IV. Magnetic labeling of the cells

One vial of (7.5mL) CliniMACS CD8 Reagent is ready to use and sufficient for one normal scale preparation as described below. For a large scale preparation, two vials of CliniMACS CD8 Reagent are required. The reagent is not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze. The reagent is to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent is printed on the reagent vial. DO NOT use the reagent after the use-by date.

Incubation with the CliniMACS® CD8 Reagent

1. Record the lot number and use-by date of the CliniMACS® CD8 Reagent(s).

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from a) one or b) two CliniMACS CD8 Reagent vials (7.5 mL each). A 10 mL syringe is sufficient to remove the contents of one vial for a normal scale application, or respectively, a 20 mL syringe is sufficient to remove the contents of two reagent vials for a large scale application. The syringe should be equipped with a 20 gauge needle.

3. Using the injection port on the sampling site coupler, inject the entire volume of reagent(s) into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

5. Place the Cell Preparation Bag flat on the orbital rotator at approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Removal of excess reagent

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 500 mL of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer. To stop the liquid flow, close the clamp on the plasma transfer set. Close the locking forceps next to the Cell Preparation Bag. Record the weight of the filled Cell Preparation Bag.

5. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag on the balance. Tare the balance.

14. Open the locking forceps next to the Cell Preparation Bag tubing. Using the plasma extractor, remove as much excess supernatant as possible from the Cell Preparation Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

15. Close the locking forceps and seal off the tubing to disconnect the Wash Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.

16. Keep the Wash Waste Bag until the separation and final analysis of all cells has been accomplished.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of leukapheresis product after the wash (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

19. Adjust sample loading volume: Calculate the amount of buffer necessary to adjust the weight of the cell suspension to approximately a) 100 g (normal scale preparation) or b) 200 g (large scale preparation). For loading the labeled and washed cells on the tubing set, a maximum cell concentration of 0.4×10^9 cells per mL is recommended.

a)	Amount of buffer to be to be added (g)	=	100 g	–	Weight of leukapheresis product after the wash (g)
b)	Amount of buffer to be to be added (g)	=	200 g	–	Weight of leukapheresis product after the wash (g)

20. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

21. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

22. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

23. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

24. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

25. Resuspend the cell pellet in the Cell Preparation Bag. Avoid too intensive mixing of the cells. Ensure that all cells are resuspended.

26. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (should include patient identification) and retain for cell analysis. WBC content, CD8 enumeration and viability testing are recommended.

27. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the addition of buffer (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of leuka-pheresis product after buffer addition (g)	=	Weight of filled Cell Pre-paration Bag (g)	–	Weight of empty Cell Pre-paration Bag (g)

Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® Cytokine Capture System (IFN-gamma)

I. General information, warnings and precautions

General information

The CliniMACS® Plus Cytokine Capture System (CCS) including the CliniMACS ^{plus} Instrument, the CliniMACS Cytokine Capture System (IFN-gamma), the CliniMACS Tubing Set TS, and CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human IFN-gamma secreting cells from heterogeneous hematologic cell populations.

Interferon gamma (IFN-gamma) is secreted by CD4 positive T cells and CD8 positive T cells upon activation with antigens. The CliniMACS Cytokine Capture System (IFN-gamma) allows for the simultaneous enrichment of both subsets of T cells needed for a sustained immune-reaction. Another source of this cytokine are natural killer cells and activated monocytes.

For the enrichment of IFN-gamma secreting cells, the CliniMACS Plus CCS System utilizes in a first step hybrid-molecules consisting of murine monoclonal antibodies specific for CD45 and Interferon gamma called the CliniMACS IFN-gamma Catchmatrix Reagent. The CliniMACS IFN-gamma Enrichment Reagent, used as second step reagent, consists of an IFN-gamma specific antibody conjugated to super-paramagnetic particles (for detailed description, see chapter 3.9).

Antigen-specific T cells can be enriched using the CliniMACS Cytokine Capture System (IFN-gamma) starting from leukapheresis harvest or PBMC. In the first step of the protocol, the cells are restimulated with specific peptide, protein or other antigen preparations in a gas-permeable culture bag for a short period of time. Subsequently, all leukocytes in the cell-suspension are labeled with the CliniMACS IFN-gamma Catchmatrix Reagent. The cells are then incubated for a short period of time at +37 °C to allow cytokine secretion. The secreted IFN-gamma is bound to the CliniMACS IFN-gamma Catchmatrix Reagent on the IFN-gamma secreting cells. These cells are labeled with the CliniMACS IFN-gamma Enrichment Reagent.

When magnetically labeled, the cells are ready for the enrichment in an automated continuous flow separation process.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

The CliniMACS Plus CCS System passes the labeled suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled cells (target cells) while unwanted cells (non-target cells) flow through the column. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged, and the magnetic field is removed. The target cells are collected in the Cell Collection Bag.

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, cellular starting product, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® Cytokine Capture System (IFN-gamma)

Intended purpose

- The CliniMACS® Cytokine Capture System (IFN-gamma) is intended for the *in vitro* enrichment of human IFN-gamma secreting cells from heterogeneous hematologic cell populations in combination with the CliniMACS System only. Upon restimulation these cells can be enriched due to their secretion of IFN-gamma.

- The CliniMACS Cytokine Capture System (IFN-gamma) is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS Cytokine Capture System (IFN-gamma), the CliniMACS Tubing Set TS, and CliniMACS PBS/EDTA Buffer.

Composition

One CliniMACS Cytokine Capture System (IFN-gamma) consists of two reagent vials:

- Each CliniMACS IFN-gamma Catchmatrix Reagent vial (7.5 mL) contains a solution of murine monoclonal anti-IFN-gamma antibody conjugated to murine monoclonal anti-CD45 antibody in PBS/EDTA buffer stabilized with Poloxamer 188 (0.03% w/v).

- Each CliniMACS IFN-gamma Enrichment Reagent vial (7.5 mL) contains a colloidal solution of iron-dextran beads conjugated to murine monoclonal anti-IFN-gamma antibody in PBS/EDTA buffer stabilized with Poloxamer 188 (0.03% w/v).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Side-effects

- When the enriched or *in vitro* expanded, IFN-gamma secreting cells are infused or injected into patients, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions are clinically used for the treatment of iron-deficiency syndroms. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease could be more susceptible to iron-dextran reactions.

Precautions

- Before use in human application, the suitability of the target cells must be demonstrated regarding indication, quality and quantity.

- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.

- Any clinical application of the separated cells is exclusively within the responsibility of the user.

- The enrichment of IFN-gamma secreting cells using the CliniMACS Cytokine Capture System (IFN-gamma) must be performed by trained operators only.

- Operators using the CliniMACS Cytokine Capture System (IFN-gamma) should have experience in the separation of cells from bone marrow or peripheral blood.

- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Important

This protocol requires the use of components which are not part of the CliniMACS Plus CCS System (e.g., HSA). Therefore, all materials must be pharmaceutical grade or FDA-licensed for human use.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

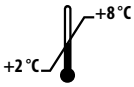


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Fig. 4.1-5: Sterilized using aseptic processing techniques.

Warnings

- For *in vitro* use only. Do not inject or infuse the reagents directly into the patient. Not for parenteral application.
- The CliniMACS Cytokine Capture System (IFN-gamma) is not recommendedfor use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The CliniMACS Cytokine Capture System (IFN-gamma) is for single use only (see fig. 4.1-1).
- Do not use the reagent after the use-by date printed on the vial label (see fig. 4.1-2).
- Do not use the reagents if package is damaged (see fig. 4.1-3). Use undamaged and sealed vials only.
- Store at +2 °C to +8 °C (see fig. 4.1-4). Do not freeze.

Further information

- The CliniMACS Cytokine Capture System (IFN-gamma) is tested for endotoxins.
- The CliniMACS Cytokine Capture System (IFN-gamma) is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

II. First actions required

CliniMACS® Materials required

- **CliniMACS® Cytokine Capture System (IFN-gamma), REF 279-01.**
  One CliniMACS Cytokine Capture System (IFN-gamma) consists of two vials of reagent, the CliniMACS IFN-gamma Catchmatrix Reagent and the CliniMACS IFN-gamma Enrichment Reagent. Each vial contains 7.5 mL of reagent.

- **CliniMACS Tubing Set TS, REF 161-01.**

- **CliniMACS PBS/EDTA Buffer, REF 700-25.**
  CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. For the preparation procedure, two liter of cold buffer (+2 °C to +8 °C) are required. For the separation, one liter of buffer (room temperature) is required. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Note

The application capacity for the enrichment of IFN-gamma secreting cells using the CliniMACS Plus CCS System amounts to a total cell number of 1×10^9 cells.

Additional materials required

- **Cell Preparation Bag***:*
  Two 600 mL transfer tags, suitable for centrifugation, as well as two sampling site couplers and two plasma transfer sets, for use during the cell preparation procedure.

- **Plasma Waste Bags and Wash Waste Bags***:*
  Nine 600 mL transfer bags, suitable for centrifugation.

- **Medium Bags***:*
  Eight 600 mL transfer bags, suitable for centrifugation.

- **Cell Collection Bag***:*
  One 150 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set.

- One 100 mL gas-permeable cell culture bag.

- Pre-system filter.

- Locking forceps.

- Appropriate syringes (1 mL, 10 mL) and hypodermic 20 gauge needles.

- Sample tubes.

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 “Additional materials required”.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Note

This protocol requires the use of components which are not part of the CliniMACS Plus CCS System (e.g.medium, AB serum). Such materials must be pharmaceutical grade or FDA-licensed for human use.

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v). Only FDA-licensed HSA should be used.
- 120 mL of AB serum or, alternatively, autologous serum, as supplement for the media used.
- 1 liter of cell culture medium (RPMI 1640), +4 °C.
- 1100 mL of cell culture medium (RPMI 1640), +37 °C, supplemented with 10% (v/v) of AB serum or, alternatively, autologous serum.
- 500 mL of cell culture medium (RPMI 1640, +4 °C, supplemented with 2% (v/v) of AB serum or, alternatively, autologous serum.

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3: "Equipment required".

Important

- Note that **HSA is not a component of the CliniMACS Plus System**. Use only FDA licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.
- Store the two bags of buffer for cell preparation cold at +2 to +8 °C. Lower or higher ambient temperature may result in less purity and yield of the target cells.

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement three liters of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v), i.e., add 5 g HSA per liter buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Labeling and preparation of bags

- Label one 150 mL transfer bag as: **Cell Collection**. (This should include patient identification, date and time of run, and operator identification.) Insert a Luer/Spike Interconnector into the port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with the locking forceps positioned close to the bag and the tubing hanging on the table next to the balance.

- Label two 600 mL transfer bags as Cell Preparation Bags for the first steps of the cell preparation: **Cell Preparation No.1 (Start) and Cell Preparation No. 2 (Secretion)**. (This should include patient identification, date and time of run, and operator identification.) Place a locking forceps on the tubing close to each bag and close the locking forceps. Weigh the empty Cell Preparation Bags with the locking forceps positioned close to the bags and the tubing hanging on the table next to the balance.

- Label two 600 mL transfer bags as: **Plasma Waste No.1** and **Plasma Waste No. 2**. (This should include patient identification, date, time of run and operator identification.)

- Label seven 600 mL transfer bags as Wash Waste Bags for the different steps of the cell preparation (This should include patient identification, date and time of run, operator identification.):
  Wash Waste No. 1 – Centrifugation for labeling with the CliniMACS IFN-gamma Catchmatrix Reagent,
  Wash Waste No. 2, Wash Waste No. 3, Wash Waste No. 4, Wash Waste No. 5, and **Wash Waste No. 6** – Secretion phase,
  Wash Waste No. 7 – Centrifugation for magnetic labeling with the CliniMACS IFN-gamma Enrichment Reagent.

- Prepare two 600 mL transfer bags, each with 500 mL of cold medium (+2 °C to +8 °C) and label the bags as: Medium.

- Prepare one 600 mL transfer bag with 600 mL of medium (RPMI) supplemented with 10% AB serum. Pre-warm at +37 °C. Label the transfer bag as: **Medium 10%**.

- Prepare one 600 mL transfer bag with 500 mL of medium (RPMI) supplemented with 2% AB serum, at +4 °C. Label the transfer bag as: **Medium 2%**.

- Prepare four 600 mL transfer bags and fill each with 125 mL of medium (RPMI) supplemented with 10% AB serum. Pre-warm at +37 °C. Label one of those bags as: **Cell Preparation No. 3 (Separation)**. (This should include patient identification, date and time of run, and operator identification.)

Note

- Pre-cool the centrifuge to +4 °C.
- After the secretion phase, a third Cell Preparation Bag is required to recombine the cell suspensions. Therefore, label one of the four 600 mL transfer bags (prepared with 125 mL of medium (RPMI) supplemented with 10% AB serum) as Cell Preparation No. 3 (Separation) as described below.
- Since the length of the tubing can vary during the preparation procedure, be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading, confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- All bag handling should be preformed in a sterile environment (e.g. laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform the sample preparation and cell separation at temperatures indicated. Lower or higher ambient temperature will result in less purity and yield of the target cells.

III. Cellular starting product

The following sections describe the recommended procedure for preparing the cellular starting product using the Terumo Sterile Connection Device (TSCD).

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Transfer of cellular starting product into Cell Preparation Bag No. 1

1. Record the date and the start time before beginning to prepare the cellular starting product.

2. Determine the volume of the original cellular product by estimating 1 mL of original cellular product as equivalent to 1 g (1 g = 1 mL).

3. Holding the bag containing the cellular starting product with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the Cell Preparation Bag No. 1 to the bag containing the cellular starting product.

5. Tare the balance. Lay the empty Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the empty Cell Preparation Bag.

6. Open the locking forceps to transfer the appropriate amount of the cellular starting product (containing a maximum of 1×10^9 cells, according to the frequency of leukocytes determined before) into the Cell Preparation Bag No. 1. Visually control (by monitoring the scale) that the appropriate amount has been transferred. Close the locking forceps.

7. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag No. 1 for further connections (see fig. 4.1-6). Retain the bag which contained the cellular starting product until cell separation and final analysis have been completed.

8. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the leukapheresis product. Transfer the sample into a sample tube. Label the tube as CELLULAR STARTING PRODUCT (should include patient identification) and retain for cell analysis. Minimally, analysis of the WBC content, viability testing and are recommended.

9. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cel Preparation Bag.

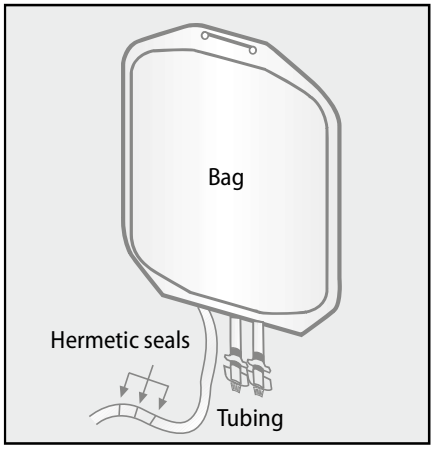


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

Note

The maximum capacity of the CliniMACS Cytokine Capture System (IFN-gamma) is 1×10^9 total cells. Remove the appropriate volume of cellular starting product.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

10. Determine the actual weight of the cellular starting product by subtracting the weight of the empty Cell Preparation Bag No. 1 from the weight of the Cell Preparation Bag No. 1 filled with the original cellular product. Record the weight.

Weight of cellular starting product (g)	=	Weight of filled Cell Preparation Bag No. 1 (g)	–	Weight of empty Cell Preparation Bag No. 1 (g)

Dilution of cellular starting product

The cellular starting product must be washed with culture medium. Dilute the product with cold medium (+2 °C to +8 °C) to a total volume of 500 mL. Calculate the amount of medium to be added using the following equation and record it.

Weight of medium to be added (g)	=	500 g	–	Weight of cellular starting product (g)

1. Take one of the Medium Bags containing cold medium and connect it to the Cell Preparation Bag No. 1 using the TSCD.

2. Place the Cell Preparation Bag No. 1 on the balance and tare the balance. Hang the Medium Bag on a bag hanger.

3. Open the locking forceps next to the Cell Preparation Bag No. 1. By visually monitoring the scale on the balance, transfer the calculated amount of medium to the Cell Preparation Bag No. 1.

4. When the appropriate amount of medium has been transferred, close the locking forceps next to the Cell Preparation Bag No. 1 to stop the liquid flow. Record the weight of the filled Cell Preparation Bag No. 1.

5. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag No. 1. Disconnect the Medium Bag.

6. Holding the Cell Preparation Bag No. 1 with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

7. Determine the total weight of the diluted cellular starting product by subtracting the weight of the empty Cell Preparation Bag No. 1 from the weight of the filled Cell Preparation Bag No. 1. Record the weight.

Weight of diluted cellular starting product (g)	=	Weight of filled Cell Preparation Bag No. 1 (g)	–	Weight of empty Cell Preparation Bag No. 1 (g)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag No. 1 to the Cell Preparation Bag No. 1.

2. Fold any loose parts of the Cell Preparation Bag No. 1 or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 200×g for 10 min (without brake) at room temperature (+19 °C to +25 °C).

5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag No. 1 onto the plasma extractor.

6. Using the plasma extractor, remove the supernatant completely. Maintain constant control of the extractor release handle and ensure the locking forceps next to the Cell Preparation Bag are in the open position before beginning the transfer of fluid. Release the extractor handle slowly being careful not to lose cells.

7. Close the locking forceps next to the Cell Preparation Bag No. 1 when the supernatant has been removed. Use the heat sealer to seal off the tubing to disconnect Plasma Waste Bag No. 1 leaving at least 15 cm of tubing on the Cell Preparation Bag No. 1 for further connections.

8. Repeat dilution and centrifugation steps described above using the second Medium Bag prepared with cold medium and the Plasma Waste Bag No. 2.

Important

- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is in the open position before beginning the transfer. Release the extractor handle slowly.

- Keep the Plasma Waste Bags until the separation and final analysis of all cells has been accomplished.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Volume adjustment

If the cells must be re-stimulated, the volume of the cell sample must be adjusted to an optimal volume according to validated procedures established by the user of the CliniMACS Cytokine Capture System (IFN-gamma). Generally, the optimal volume of the cell sample is 100 mL, with a cell density of 0.5-1x10^7 cells/cm^2 and 1x10^7 cells/mL

1. For *in vitro* restimulation of the cells, the volume of the cell sample must be adjusted to 100 mL. When the supernatant has been removed, resuspend the pellet carefully.

2. Using the TSCD, connect the Cell Preparation Bag No. 1 to the bag labeled Medium 10% (containing 600 mL pre-warmed culture medium supplemented with 10% AB serum).

3. Place the Cell Preparation Bag No. 1 containing the cell pellet on the balance. Adjust the weight of the Cell Preparation Bag No. 1 by adding the appropriate amount of culture medium. Calculate the amount of medium to be added using the equation below:

Weight of filled Cell Preparation Bag No. 1 after volume adjustment (g)	=	100 g	+	Weight of emtpy Cell Preparation Bag No. 1 (g)

4. When the appropriate amount of medium has been transferred, close the locking forceps next to the Cell Preparation Bag No. 1 to stop the liquid flow.

5. Using the heat sealer, seal off the tubing to disconnect the bag labeled Medium 10%.

6. Using the TSCD, connect the filled Cell Preparation Bag No. 1 to a 100 mL gas-permeable culture bag, open the locking forceps, and completely transfer the content.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

IV. Labeling of cells

One CliniMACS Cytokine Capture System (IFN-gamma) comprised of one vial of CliniMACS IFN-gamma Catchmatrix Reagent and one vial of IFN-gamma Enrichment Reagent. Each vial contains 7.5 mL of reagent and is ready to use and sufficient for one application as described below. The reagents are not for parenteral administration.

Store the reagents at +2 °C to +8 °C. DO NOT freeze. The reagents are to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagents are printed on the reagent vial. DO NOT use the reagents after the use-by date.

In vitro restimulation

If *in vitro* re-stimulation is necessary, this is always performed according to validated procedures established by the user of the CliniMACS Cytokine Capture System (IFN-gamma). Generally, re-stimulation is performed using appropriate cell culture medium with a usual volume of the cell sample of 100 ml, and a cell dencity of 0.5-1x10^7 cells/cm^2 and 1x10^7 cells/mL.

Labeling with the CliniMACS® IFN-gamma Catchmatrix Reagent

Record the lot number and use-by date of the CliniMACS® IFN-gamma Catchmatrix Reagent.

1. Using the TSCD, connect the culture bag to the Cell Preparation Bag No. 2 (Secretion) and transfer the cells. Ensure the complete transfer of the cells into the Cell Preparation Bag No. 2. Close the locking forceps next to the Cell Preparation Bag No. 2 and use the heat sealer to seal off the tubing to disconnect the empty culture bag.

2. Connect the bag labeled Medium 2% (containing 500 mL cold (+2 to +8 °C) culture medium supplemented with 2% AB serum) to the Cell Preparation Bag No. 2. Open the locking forceps next to the Cell Preparation Bag No. 2 and fill the Cell Preparation Bag No. 2 up to 600 mL.

3. When the appropriate amount of medium has been transferred, close the locking forceps next to the Cell Preparation Bag No. 2 and use the heat sealer to seal off the tubing to disconnect the Medium Bag 2%.

Important

For the secretion phase, the vessel of choice must be of sufficient size to allow the addition of at least 1 volume of cold buffer at the end of the secretion phase. The cell density must not exceed 1×10^6 cells/mL. Therefore, 1×10^9 cells must be incubated for secretion in a total of 1000 mL of warm culture medium.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

4. Using the TSCD, connect the empty Wash Waste Bag No. 1 to the Cell Preparation Bag No. 2. Fold any loose parts of the bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

5. Balance the loaded bucket with a suitably weighed bucket. It is essential that the centrifuge is balanced accurately.

6. Centrifuge cells at 300×g for 10 minutes without brake at +4 °C to +8 °C.

7. Place the Cell Preparation Bag No. 2 on the plasma extractor, open the locking forceps and completely remove the supernatant, taking care not to disturb the cell pellet.

8. Close the locking forceps and using the heat sealer, seal off the tubing to disconnect the Wash Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag No. 2 for further connections.

9. Resuspend the cell pellet in the residual volume after removal of the supernatant.

10. Attach a sampling site coupler to the Cell Preparation Bag No. 2 and disinfect the septum. Use an appropriate sterile syringe and needle to remove 7.5 mL from the CliniMACS IFN-gamma Catchmatrix Reagent vial. A 10 mL syringe is sufficient to remove the contents of one reagent vial. The syringe should be equipped with a 20 gauge needle.

11. Holding the Cell Preparation Bag No. 2 with both hands, mix the contents thoroughly by using a gentle rotating motion.

12. Using the injection port on the sampling site coupler, inject the entire volume of the CliniMACS IFN-gamma Catchmatrix Reagent into the Cell Preparation Bag No. 2. Take care not to puncture the bag.

13. Mix the cells and incubate them for 5 minutes on ice. Record the incubation time.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Secretion phase

If necessary, the cells must be cultured for secreting IFN-gamma according to validated procedures established by the user of the CliniMACS Cytokine Capture System (IFNgamma). Generally, the cells are incubated for 45 min at 37 °C in suitable cell culture medium containing 10% AB serum and at a cell desity of approximatly 1×10^6 cell/ml.

1. Take the four pre-filled transfer bags (including the labeled Cell Preparation Bag No. 3) containing 125 mL of warm (+37 °C) culture medium supplemented with 10% AB serum.

2. Using the TSCD, connect the Cell Preparation Bag No. 2 to the bag labeled as Medium 10% (containing pre-warmed (+37 °C) culture medium supplemented with 10% AB serum).

3. Add 500 mL of the pre-warmed culture medium to the cell sample in the Cell Preparation Bag No. 2. Begin recording the 45 minute incubation time immediately following the addition of the 500 mL of the pre-warmed culture medium.

4. Distribute 125 mL of the resulting cell suspension in each of the four bags by serial attachment of the bags to the Cell Preparation Bag No. 2 using the TSCD. By visually monitoring the scale on the balance, transfer the appropriate amount of cell suspension and mix gently. This procedure results in a total of 250 mL of cell suspension in each of the four bags. After the transfer, use the heat sealer to seal off the tubing and disconnect the bags.

5. Incubate the cells at +37 °C for the remainder of the 45 minutes with continuous slow rotation (max 50 rpm) until the end of incubation time. Record the incubation time.

6. Take a plasma transfer set and ensure that the clamp is closed. Insert the spike of the plasma transfer set into a port of the buffer bag.

7. After the 45 minutes, connect the cold CliniMACS PBS/EDTA buffer (+2 °C to +8 °C) bag to the Cell Preparation Bag No. 3 using the TSCD. Add the cold buffer to each of the four bags (at least one volume i.e. 250 mL each). Allow the cells to cool down by placing the bags on ice for 10 minutes.

8. Using the TSCD, connect Wash Waste Bags to each of the four bags (Wash Waste Bags Nos. 2 to 5).

9. Centrifuge cells at 300×g without brake for 10 minutes at +4 °C and completely remove supernatant from all of the bags using the plasma extractor.

Important

Begin recording the 45 minutes incubation time immediately following addition of the 500 mL warm culture medium.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

10. Using the heat sealer and seal off the tubing of each bag to disconnect the corresponding Wash Waste Bag.

11. Take a plasma transfer set and ensure that the clamp is closed. Insert the spike of the plasma transfer set into a port of a buffer bag. Using the TSCD, connect the cold CliniMACS PBS/EDTA Buffer (+2 °C to +8 °C) bag to the Cell Preparation Bag No. 3. Open the clamp of the plasma transfer set und fill the Cell Preparation Bag No. 3 with 200 mL of cold buffer.

12. When the appropriate amount of buffer is transferred, close the clamp on the plasma transfer set to stop the liquid flow. Use the heat sealer to seal off the tubing and disconnect the buffer bag.

Important

The divided samples have to be recombined in the Cell Preparation Bag No. 3 before centrifugation.

13. The divided samples must now be transferred to the Cell Preparation Bag prior to centrifugation. Using the TSCD, attach one of the other three transfer bags containing the cells and to the Cell Preparation Bag. Open the weld and allow the flow suspension to flow into the Cell preparation bag.

14. Repeat this procedure until the cell suspensions from all four transfer bags are recombined in the bag labeled as Cell Preparation Bag No. 3.

15. After the cells have been transferred to the Cell Preparation Bag, connect the cold CliniMACS PBS/EDTA Buffer (+2 °C to +8 °C) bag to the Cell Preparation Bag No. 3. Open the clamp of the plasma transfer set and fill the Cell Preparation Bag with up to 500 mL of buffer.

16. When the appropriate amount of buffer is transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag No. 3 and use the heat sealer to seal off the tubing to disconnect the buffer bag.

17. Using the TSCD, connect the empty Wash Waste Bag No. 6 to the Cell Preparation Bag No. 3.

18. Fold any loose parts of the Cell Preparation Bag No. 3 or tubing downwards. Place the two bags securely in the centrifuge bucket.

19. Balance the loaded bucket with a suitably weighed bucket. It is essential that the centrifuge is balanced accurately.

20. Centrifuge cells at 300×g without brake for 10 minutes at +4 °C.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Magnetic labeling of cells with the CliniMACS® IFN-gamma Enrichment Reagent

Record the lot number and use-by date of the CliniMACS® IFN-gamma Enrichment Reagent.

1. After the centrifugation step, carefully remove supernatant using the plasma extractor, as described above and resuspend the cell pellet in the residual volume. Close the locking forceps next to the Cell Preparation Bag No. 3 after removal and and heat seal off the tubing to disconnect the Waste Waste Bag No. 6.

2. Attach a sampling site coupler to the Cell Preparation Bag No. 3 and disinfect the septum. Use an appropriate sterile syringe and needle to remove 7.5 mL from the CliniMACS IFN-gamma Enrichment Reagent vial. A 10 mL syringe is sufficient to remove the contents of one reagent vial. The syringe should be equipped with a 20 gauge needle.

3. Using the injection port on the sampling site coupler, inject the reagent into the Cell Preparation Bag No. 3. Take care not to puncture the bag. Immediately begin the 15 minute incubation. Record the incubation start time.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

5. Incubate the cells for 15 minutes on ice. Record the incubation stop time.

6. Using the TSCD, connect the cold CliniMACS PBS/EDTA Buffer (+2 °C to +8 °C) bag to the Cell Preparation Bag No. 3. Open the locking forceps next to the Cell Preparation Bag No. 3 and fill the Cell Preparation Bag No. 3 up to 500 mL with the cold buffer.

7. When the appropriate amount of buffer has been transferred, close the locking forceps next to the Cell Preparaton Bag No. 3 and using the heat sealer, seal off the tubing to disconnect the buffer bag.

8. Using the TSCD, connect the empty Wash Waste Bag No. 7 to the Cell Preparation Bag No. 3. Fold any loose parts of the bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

9. Balance the loaded bucket with a suitably weighed bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g without brake for 10 minutes at +4 °C.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

11. Carefully remove the supernatant as described above and resuspend the cells in 100 mL of cold (+2 °C to +8 °C) CliniMACS PBS/EDTA Buffer.

12. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Take care not to puncture the bag. Transfer the sample into a sample tube. Label the tube as ORIGINAL TARGET SAMPLE (should include patient identification). WBC content, viability testing and are recommended.

13. Retain the cell sample for cell analysis stored in the cold (+2 °C to +8 °C).

Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® CD25 Reagent

I. General information, warnings and precautions

General information

The CliniMACS® Plus CD25 System including the CliniMACS ^{plus} Instrument, the CliniMACS CD25 Reagent, the CliniMACS Tubing Set TS or a CliniMACS Tubing Set LS, and CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment or depletion of human CD25 positive cells -preferably CD25 highly expressing cells- from heterogeneous hematologic cell populations.

The CD25 antigen, the low affinity interleukin-2 receptor alpha chain (IL-2R alpha), is expressed on activated T and B cells, NK cells and monocytes as well as on regulatory CD4 positive cells. The CD25 antigen shows three epitope regions A, B and C. The antibody of the CliniMACS CD25 Reagent recognizes epitope A of the CD25 molecule.

The CliniMACS Plus CD25 System uses selective CD25 monoclonal antibodies conjugated to super-paramagnetic particles. The CD25 positive cells are specifically labeled by incubation with the CliniMACS CD25 Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the separation in an automated continuous flow separation process.

For CD25 positive cells either an enrichment or a depletion strategy can be chosen. For enrichment of CD25 positive or CD25 highly expressing cells the separation program ENRICHMENT 3.2 must be chosen. For depletion of CD25 positive cells the separation program DEPLETION 2.1 must be chosen.

The CliniMACS Plus CD25 System passes the antibody-labeled suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled cells while the non-labeled cells flow through the separation column. Several washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged, and the magnetic field is removed. The target cells are collected in the Cell Collection Bag. Using a depletion strategy, the separation column retains the magnetically labeled CD25 positive cells (non-target cells), while the unlabeled cells (target cells) flow through the column. Several automated washing steps are performed and the CD25 negative target cells are collected in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, cellular starting product, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® CD25 Reagent

Intended purpose

- The CliniMACS® CD25 Reagent is intended for the *in vitro* enrichment or depletion of human CD25 positive cells from heterogeneous hematologic cell populations in combination with the CliniMACS System only.

- The CliniMACS Plus CD25 System is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS CD25 Reagent, the CliniMACS Tubing Set TS or CliniMACS Tubing Set LS, and CliniMACS PBS/EDTA Buffer.

Composition

Each CliniMACS CD25 Reagent vial contains 7.5 mL of a colloidal solution of iron-dextran beads conjugated to murine monoclonal CD25 antibody in PBS/EDTA buffer stabilized with Poloxamer 188 (0.03% w/v).

Side-effects

- When the CD25 enriched or depleted cell fraction is infused or injected into patients, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndromes. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease could be more susceptible to iron-dextran reactions.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Precautions

- Before use in human applications, the suitability of the target cells must be demonstrated regarding indication, quality and quantity.
- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively within the responsibility of the user.
- The separation of CD25 positive cells using the CliniMACS CD25 Reagent must be performed by trained operators only.
- Operators using the CliniMACS CD25 Reagent should have experience in the separation of cells from bone marrow or peripheral blood.
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- For *in vitro* use only. Do not inject or infuse the reagent directly into the patient. Not for parenteral application.
- The CliniMACS CD25 Reagent is not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The CliniMACS CD25 Reagent is for single use only (see fig. 4.1-1).
- Do not use the reagent after the use-by date printed on the vial label (see fig. 4.1-2).
- Do not use the reagent if package is damaged (see fig. 4.1-3). Use undamaged and sealed vials only.
- Store at +2 °C to +8 °C (see fig. 4.1-4). Do not freeze.

Further information

- The CliniMACS CD25 Reagent is tested for endotoxins.
- The reagent is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

Important

This protocol requires the use of components which are not part of the CliniMACS Plus CD25 System (e.g., HSA). Therefore, all materials used must be pharmaceutical grade or FDA-licensed for human used.

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

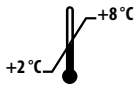


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Fig. 4.1-5: Sterilized using aseptic processing techniques.

II. First actions required

Note

- The application capacity for the **enrichment of CD25 positive cells** using the CliniMACS Plus CD25 System amounts up to 0.6×10^9 CD25 positive cells out of a total cell number not exceeding 40×10^9.

- The application capacity for the **enrichment of CD25 highly expressing cells** using the CliniMACS Plus CD25 System amounts up to 0.6×10^9 CD25 positive cells out of a total cell number not exceeding 40×10^9.

- The application capacity for **the depletion of CD25 positive cells** amounts to 6×10^9 CD25 positive cells out of a total cell number not exceeding 40×10^9 (normal scale application using one vial of CliniMACS CD25 Reagent). For the depletion of up to 12×10^9 CD25 positive cells out of a total cell number of 80×10^9, two vials of CliniMACS CD25 Reagent are needed.

CliniMACS® Materials required

- **CliniMACS® CD25 Reagent, REF 274-01.**
  Using the CliniMACS Plus CD25 System, one vial is required for a normal scale application and two vials for a large scale application (depletion).

- **CliniMACS Tubing Set TS, REF 161-01**
  Enrichment strategy (ENRICHMENT 3.2):
  A CliniMACS Tubing Set TS must be used for the enrichment of CD25 positive cells or CD25 highly expressing cells.
  o r
  CliniMACS Tubing Set LS, REF 162-01
  Depletion Strategy (DEPLETION 2.1):
  A CliniMACS Tubing Set LS must be used for the depletion of CD25 positve cells.

- **CliniMACS PBS/EDTA Buffer, REF 700-25.**
  The CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. For the preparation procedure, up to two liters of buffer are required. For the separation, up to two liters of buffer is required (see tables 4.1-1 and 4.1-2). Please note, for the enrichment of CD25 highly expressing cells store 1 liter CliniMACS PBS/EDTA Buffer at +4 °C to 8 °C. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Additional materials required

- **Cell Preparation Bag:**
  One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and one to two plasma transfer sets, for use during the cell preparation procedure.

- **Plasma Waste Bag and Wash Waste Bag:**
  Two 600 mL (or two 1000 mL) transfer bags, suitable for centrifugation.

- **Cell Collection Bag (see table 4.1-2):**
  One 150 mL or one to two 600 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set. If two Cell Collection Bags are required for the separation, use a plasma transfer set to connect both bags.

- **If the replacement of the Negative Fraction Bag and the Buffer Waste Bag is necessary (see table 4.1-2):**
  Two 1000 mL transfer bags in combination with two Luer/Spike Interconnectors to connect the bags to the tubing set.

- **If more than one liter of buffer is required for the separation (see table 4.1-2):**
  One plasma transfer set to connect the buffer bags.

- Pre-system filter.

- Locking forceps.

- Appropriate syringes (1 mL, 10 mL, 20 mL) and hypodermic 20 gauge needles.

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).

- Sample tubes.

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3: "Equipment required".

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 "Additional materials required".

Important

After input of sample parameters (STEP 2), the CliniMACS Plus Software calculates the volumes that will be collected in the Priming Waste Bag, Cell Collection Bag, Negative Fraction Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volumes the bags need to be exchanged according to table 4.1-1 and 4.1-2.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Overview of materials required

Cell preparation				
Separation	**Enrichment of CD25 positive cells**	**Enrichment of CD25 highly expressing cells**	**Depletion of CD25 positive cells**	
Total cells (WBC)	≤40×10^9	≤40×10^9	≤40×10^9	40–80×10^9
Labeled cells	≤0.6×10^9	≤0.6×10^9	≤6×10^9	6–12×10^9
Number of CliniMACS CD25 Reagent vials	1	1	1	2
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1–2 liters	2 liters	1–2 liters	1–2 liters
Plasma transfer set	1–2	2	1–2	1–2
Labeling volume	190 mL	380 mL	95 mL	190 mL
Labeling temperature	room temperature	+4°C to +8°C	room temperature	room temperature

Table 4.1-1: Materials required for enrichment or depletion of the cells during the cell preparation.

CliniMACS Plus Separation				
Separation	**Enrichment of CD25 positive cells**	**Enrichment of CD25 highly expressing cells**	**Depletion of CD25 positive cells**	
Total cells (WBC)	≤40×10^9	≤40×10^9	≤40×10^9	40–80×10^9
Tubing set	1 CliniMACS Tubing Set TS (REF 161-01)	1 CliniMACS Tubing Set TS (REF 161-01)	1 CliniMACS Tubing Set LS (REF 162-01)	
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter for ≤0.6×10^9 labeled cells	1 liter for ≤0.6×10^9 labeled cells	1 liter for <5×10^9 labeled cells	2 liters for 5–12×10^9 labeled cells
Negative Fraction Bag	–	–	–	Replace original bags of the tubing set by **1000 mL** transfer bags
Buffer Waste Bag				
Priming Waste Bag				–
Cell Collection Bag	150 mL	150 mL	600 mL	2× 600 mL
Luer/Spike Interconnector	1	1	1	3
Plasma transfer set	–	–	–	2

Table 4.1-2: Materials required for enrichment or depletion of the cells during the CliniMACS Plus Separation.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement each required liter of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5 % (w/v), i.e., add 5 g HSA per liter buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

- Label one (or two) transfer bag(s) (see tables 4.1-1 and 4.1-2) as: **Cell Collection**. (Labeling should include patient identification, date and time of run, and operator identification.) Insert a Luer/Spike Interconnector into the outside port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL transfer bag as: **Cell Preparation**. (This should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL (or 1000 mL) Transfer Bag as: **Plasma Waste**. (This should include patient identification, date, time of run and operator identification.)

- Label one 600 mL (or 1000 mL) Transfer Bag as: **Wash Waste**. (This should include patient identification, date and time of run, operator identification.)

Important

- Note that **HSA is not a component of the CliniMACS System**. Use only FDA licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.

- **Store the buffer for cell preparation at +19 °C to +25 °C.**

  Magentic labeling during the cell preparation for the enrichment of CD25 highly expressing cells has to be performed using cold buffer (+4 °C to +8 °C).

  Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the tubing can vary during the preparation procedure be careful when determining the weight of the Cell Preparation Bag. To ensure an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- All bag handling should be preformed in a sterile environment (e.g. laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform **sample preparation for the enrichment or the depletion of CD25 positive cells at room temperature (+19 °C to +25 °C).** Perform **magnetic labeling for the enrichment of CD25 highly expressing cells at +4 °C to +8 °C.** Cells are separated at room temperature. Lower or higher temperature will result in less purity and yield of the target cells.

Note

Alternative starting cell sources can be heterogeneous hematologic cell populations such as CliniMACS depleted target cell fraction or cultured cells.

III. Leukapheresis product

The following sections describe the recommended procedure for the preparation of the leukapheresis product using the Terumo Sterile Connection Device (TSCD).

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all necessary supplies and equipment are available.

Analysis of leukapheresis product

Before starting the preparation of the leukapheresis product at least the following parameters should be determined:

- Volume and cell concentration to determine the total number of leukocytes,
- Percentage of CD25 positive cells or CD25 highly expressing cells,
- Total number of CD25 positive cells or CD25 highly expressing cells,
- Viability.

Recheck these parameters after the labeling procedure. This is necessary because the following three values will be required during the setup of the CliniMACS ^{plus} Instrument prior to installing the tubing set on the instrument (only for use of separation program DEPLETION 2.1, no sample parameter input is required when using ENRICHMENT 3.2):

- Concentration of leukocytes per mL,
- Percentage of CD25 positive cells,
- Final volume of the cell sample (sample loading volume).

Other tests might be required depending on the intended use of the cells or individual laboratory practice.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Transfer leukapheresis product into Cell Preparation Bag

1. Record the date and the start time before beginning to prepare the leukapheresis product.

2. Determine the volume of the original leukapheresis product by estimating 1 mL of leukapheresis product as equivalent to 1 g (1 g = 1 mL).

3. Holding the leukapheresis product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the Cell Preparation Bag to the original leukapheresis product bag.

5. Open the locking forceps to transfer the leukapheresis product material into the Cell Preparation Bag. Use a tubing stripper to clear the tubing from any remaining blood. Close the locking forceps.

6. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see fig. 4.1-6). Retain the original leukapheresis bag until separation and final analysis have been completed.

7. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the leukapheresis product. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS PRODUCT (should include patient identification) and retain for cell analysis. Analysis of the WBC content, CD25 content and viablity testing are recommended.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the actual weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with leukapheresis product. Record the weight.

Weight of leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

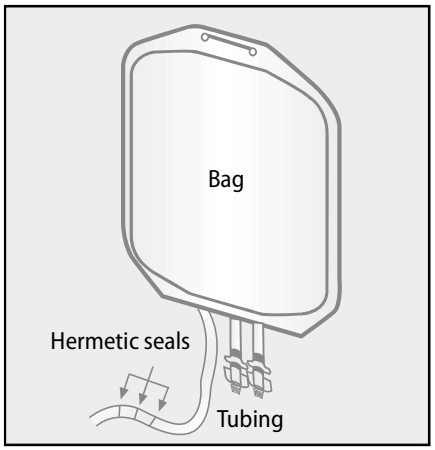


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Note

If the CliniMACS depleted target cell fraction (alternative starting cell source) is below or equal to 190 mL or 380 mL respectively please continue with "Volume adjustment" on page 11.

Dilution of leukapheresis product

The leukapheresis product must be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Dilute the leukapheresis product to a total volume of three times the volume of the leukapheresis product, but not exceeding a total volume of 600 mL. Calculate the amount of buffer to be added using the following equation and record it.

Weight of buffer to be added (g)	=	Weight of leuka-pheresis product (g)	×	2

1. Take a plasma transfer set and ensure the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

4. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

9. Determine the weight of the diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of diluted leukapheresis product (g)	=	Weight of filled Cell Pre paration Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Volume adjustment

1. For magnetic labeling of CD25 positive cells, the weight of the cell sample must be adjusted.

   For the enrichment of CD25 positive cells the optimal labeling volume is a) 190 g (± 5 g) in buffer at room temperature using one vial of CliniMACS CD25 Reagent.

   For the enrichment of CD25 highly expressing cells the optimal labeling volume is b) 380 g (± 5 g) in cold buffer (+4 °C to +8 °C) using one vial of CliniMACS CD25 Reagent.

   For the depletion strategy the optimal labeling volume is c) 95 g (± 5 g) if one vial of CliniMACS CD25 Reagent is sufficient or d) 190 g (± 5 g) if two vials of reagent are needed. Keep the buffer at room temperature.

   Remove supernatant completely, taking care not to resuspend the cell pellet during removal of supernatant. Close the locking forceps next to the Cell Preparation Bag, when the supernatant has been removed. Using the TSCD, seal off the tubing to disconnect the Plasma Waste Bag. Resuspend the pellet carefully.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is in the open position before beginning the transfer. Release the extractor handle slowly.
- During removal of supernatant be careful not to lose cells.

Place the Cell Preparation Bag with the cell pellet on the balance. Adjust the weight of the Cell Preparation Bag by adding buffer. Calculate the target weight of the Cell Preparation Bag (CPB) filled with diluted leukapheresis product (LP) using the equation below:

a)	Target weight of CPB filled with diluted LP (g)	=	190 g	+	Weight of empty CPB (g)
b)	Target weight of CPB filled with diluted LP (g)	=	380 g	+	Weight of empty CPB (g)
c)	Target weight of CPB filled with diluted LP (g)	=	95 g	+	Weight of empty CPB (g)
d)	Target weight of CPB filled with diluted LP (g)	=	190 g	+	Weight of empty CPB (g)

2. Insert the spike of a plasma transfer set to a port of a buffer bag. Confirm the clamp on the plasma transfer set is closed.

3. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

4 Tare the balance. Place the Cell Preparation Bag with the cell pellet on the balance.

5. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and fill the Cell Preparation Bag with buffer until the calculated "Target weight of Cell Preparation Bag filled with diluted leukapheresis product" is reached. To stop the liquid flow, close the clamp on the plasma transfer set. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

8. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

9. Determine the weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

10. Keep the Plasma Waste Bag until the separation and final analysis of all cells has been completed.

IV. Magnetic labeling of the cells

The CliniMACS CD25 Reagent vial (7.5 mL) is ready for use and sufficient for an application as described below. The reagent is not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze. The reagent is to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by-date and lot number of the reagent is printed on the reagent vial. DO NOT use the reagent after the use-by date.

Incubation with the CliniMACS® CD25 Reagent

1. Record the lot number and use-by date of the CliniMACS® CD25 Reagent.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from either one or two CliniMACS CD25 Reagent vials (7.5 mL each). A 10 mL syringe is sufficient to remove the contents of one reagent vial or a 20 mL syringe is sufficient to remove the contents of two reagent vials. The syringe should be equipped with a 20 gauge needle.

3. Using the injection port on the sampling site coupler, inject the entire volume of reagent into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation (see pt. 5).

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

5. Place the Cell Preparation Bag flat on the rotator at approximately 25 rpm and ensure that the bag is not creased or bent.

a) For the **enrichment or depletion of CD25 positive cells** incubate the bag for a total of **30 minutes at controlled room temperature (+19 °C to +25 °C).**

b) For the **enrichment of CD25 highly expressing cells** incubate the bag for a total of **15 minutes at +4 °C to +8 °C.**

Record the incubation stop time.

Removal of excess reagent

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 500 mL of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer. To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of the filled Cell Preparation Bag.

5. Close the locking forceps next to the Cell Preparation Bag. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

Important

If the removed supernatant is less than 500 mL, a second washing step is recommended. Otherwise the removal of unbound reagent may be insufficient.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag on the balance. Tare the balance.

14. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much supernatant as possible from the Cell Preparation Bag.

15. Close locking forceps and using the heat sealer, seal off the tubing to disconnect the Wash Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.

16. Keep the Wash Waste Bag until the separation and final analysis of all cells has been accomplished.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of leukapheresis product after the wash (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

19. **Adjust sample loading volume:**
Calculate the amount of buffer necessary to adjust the weight of the cell suspension to approximately a) 100 g (normal scale application) or b) 200 g (large scale application). For loading the labeled and washed cells on the tubing set, a maximum cell concentration of 0.4×10^9 cells per mL is recommended.

a)	Amount of buffer to be to be added (g)	=	100 g	–	Weight of leukapheresis product after the wash (g)
b)	Amount of buffer to be to be added (g)	=	200 g	–	Weight of leukapheresis product after the wash (g)

20. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

21. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

22. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

23. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

24. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

25. Resuspend the cell pellet in the Cell Preparation Bag. Avoid too intensive mixing of the cells. Ensure that all cells are resuspended.

26. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (should include patient identification) and retain for cell analysis. WBC content, CD25 enumeration and viability testing are recommended.

27. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the addition of buffer (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of leukaphe-resis product after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® CD4 Reagent

I. General information, warnings and precautions

General information

The CliniMACS® Plus CD4 System including the CliniMACS ^{plus} Instrument, the CliniMACS CD4 Reagent, the CliniMACS Tubing Set TS or a CliniMACS Tubing Set LS, and CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment or depletion of human CD4 positive cells from heterogeneous hematologic cell populations.

The CD4 antigen is an accessory molecule in the recognition of foreign antigens in association with MHC class II molecules by T cells. In addition, CD4 is a receptor for HIV-1. T helper cells and at lower level monocytes, macrophages and dendritic cells, also express CD4.

The CliniMACS Plus CD4 System uses selective CD4 monoclonal antibodies conjugated to super-paramagnetic particles. The CD4 positive cells are specifically labeled by incubation with the CliniMACS CD4 Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the separation in an automated continuous flow separation process.

For CD4 positive cells either an enrichment or a depletion strategy can be chosen. For the enrichment of CD4 positive cells, the separation program ENRICHMENT 1.1 must be used. The separation program DEPLETION 2.1 must be chosen for the depletion of CD4 positive cells.

The CliniMACS Plus CD4 System passes the antibody-labeled cell suspension through the separation column in which strong magnetic gradients are generated. Using an enrichment strategy, the separation column retains the magnetically labeled CD4 positive cells, while the unlabeled cells flow through the column. Several automated washing steps are performed. The magnetically labeled cells are released from the column when the magnet is disengaged, and the magnetic field is removed. The target cells are collected in the Cell Collection Bag. Using a depletion strategy, the separation column retains the magnetically labeled CD4 positive cells (non-target cells), while the unlabeled cells (target cells) flow through the column. Several automated washing steps are performed and the CD4 negative target cells are collected in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, cellular starting product, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® CD4 Reagent

Intended purpose

- The CliniMACS® CD4 Reagent is intended for the *in vitro* enrichment or depletion of human CD4 positive cells from heterogeneous hematologic cell populationsin combination with the CliniMACS System only.

- The CliniMACS Plus CD4 System is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS CD4 Reagent, the CliniMACS Tubing Set TS or CliniMACS Tubing Set LS, and CliniMACS PBS/EDTA Buffer.

Composition

Each CliniMACS CD4 Reagent vial contains 7.5 mL of a colloidal solution of iron-dextran beads conjugated to murine monoclonal CD4 antibody in PBS/EDTA buffer stabilized with Poloxamer 188 (0.03% w/v).

Side-effects

- When the CD4 enriched or depleted cell fraction is infused or injected into patients, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndromes. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease could be more susceptible to iron-dextran reactions.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Precautions

- Before use in human applications, the suitability of the target cells must be demonstrated regarding indication, quality and quantity.
- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively within the responsibility of the user.
- The separation of CD4 positive cells using the CliniMACS CD4 Reagent must be performed by trained operators only.
- Operators using the CliniMACS CD4 Reagent should have experience in the separation of cells from bone marrow or peripheral blood.
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- CliniMACS CD4 Reagent is for *in vitro* use only. Do not inject or infuse the reagents directly into the patient. Not for parenteral application.
- The reagent is not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The reagent is for single use only (see fig. 4.1-1).
- Do not use the reagent after the use-by date printed on the vial label (see fig. 4.1-2).
- Do not use the reagent if package is damaged (see fig. 4.1-3). Use undamaged and sealed vials only.
- Store at +2 °C to +8 °C (see fig. 4.1-4). Do not freeze.

Further information

- The CliniMACS CD4 Reagent is tested for endotoxins.
- The reagent is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

Important

This protocol requires the use of components which are not part of the CliniMACS Plus CD4 System (e.g., HSA). Therefore, all materials used must be pharmaceutical grade or FDA-licensed for human use.

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

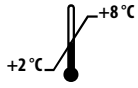


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Fig. 4.1-5: Sterilized using aseptic processing techniques.

II. First actions required

Note

- The application capacity for **the enrichment of CD4 positive cells** using the CliniMACS Plus CD4 System amounts to 5×10^9 CD4 positive cells out of a total cell number not exceeding 40×10^9 (normal scale preparation using one vial CliniMACS CD4 Reagent).
- The application capacity for **the depletion of CD4 positive cells** amounts up to 12×10^9 CD4 positive cells out of a total cell number not exceeding 40×10^9 (normal scale application using one vial of CliniMACS CD4 Reagent).
- For the depletion of up to 20×10^9 CD4 positive cells out of a total cell number of 80×10^9 (large scale application), two vials CliniMACS CD4 Reagent are needed.

CliniMACS® Materials required

- **CliniMACS® CD4 Reagent, REF 276-01.**
  Using the CliniMACS Plus CD4 System, one vial is required for a normale scale application und two vials for a large scale application (depletion).

- **CliniMACS Tubing Set TS, REF 161-01**
  Enrichment Strategy (ENRICHMENT 1.1):
  A CliniMACS Tubing Set TS must be used for the enrichment of CD4 positve cells.
  o r
  CliniMACS Tubing Set LS, REF 162-01.
  Depletion Strategy (DEPLETION 2.1):
  A CliniMACS Tubing Set LS must be used for the depletion of CD4 positve cells.

- **CliniMACS PBS/EDTA Buffer, REF 700-25.**
  The CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. The amount of buffer needed for both steps varies, depending on the selected separation program and the number of labeled cells (see table 4.1-1 and 4.1-2). Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Additional materials required

- **Cell Preparation Bag:**
  One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure.

- **Plasma Waste Bag and Wash Waste Bag:**
  Two 600 mL transfer bags, suitable for centrifugation.

- **Cell Collection Bag (see tables 4.1-1 and 4.1-2):**
  One 150 mL or one to two 600 mL transfer bags in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set. If two Cell Collection Bags are required for the separation, use a plasma transfer set to connect both bags.

- **If the replacement of the Negative Fraction Bag, the Buffer Waste Bag, and/or the Priming Waste Bag is necessary (see tables 4.1-1 and 4.1-2):**
  Two 1000 mL transfer bags in combination with two Luer/Spike Interconnectors to connect the bags to the tubing set. Replace the Priming Waste Bag using the TSCD (see "Equipment required").

- **If more than one liter buffer is required for the separation:**
  One to two plasma transfer sets to connect the buffer bags.

- Pre-system filter.

- Locking forceps.

- Appropriate syringes (1 mL, 10 mL, 20 mL or 50 mL) and hypodermic 20 gauge needles.

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).

- Sample tubes.

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3: "Equipment required".

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 "Additional materials required".

Important

After input of sample parameters (STEP 2), the CliniMACS Plus Software calculates the volumes that will be collected in the Priming Waste Bag, Cell Collection Bag, Negative Fraction Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volumes bags need to be exchanged according to table 4.1-1 and 4.1-2.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Overview of materials required

ENRICHMENT 1.1: Cell preparation for the enrichment of CD4 positive cells		
Total cells (WBC)	40×10^9	
Labeled cells	$<5\times10^9$	
Number of CliniMACS CD4 Reagent vials	1	
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	
Plasma transfer set	1	
ENRICHMENT 1.1: CliniMACS Plus Separation using the CliniMACS Tubing Set TS (REF 161-01)		
Labeled cells	$<2\times10^9$	$2–5\times10^9$
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	2 liters
Negative Fraction Bag	-	Replace original bags of the tubing set by **1000 mL** transfer bags
Buffer Waste Bag		
Priming Waste Bag		-
Cell Collection Bag	150 mL	600 mL
Luer/Spike Interconnector	1	3
Plasma transfer set	-	1

Table 4.1-1: Materials required for the enrichment of CD4 positive cells using separation program ENRICHMENT 1.1.

DEPLETION 2.1: Cell preparation for depletion of CD4 positive cells		
Total cells (WBC)	40×10^9	80×10^9
Labeled cells	$<12\times10^9$	$<20\times10^9$
No. of CliniMACS CD4 Reagent vials	1	2
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	1 liter
Plasma transfer set	1	1

DEPLETION 2.1: CliniMACS Plus Separation using the CliniMACS Tubing Set LS (REF 162-01)			
Labeled cells	$<5\times10^9$	$5–13\times10^9$	$13–20\times10^9$
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	2 liters	3 liters
Negative Fraction Bag	-	Replace original bags of the tubing set by **1000 mL** transfer bags	Replace original bags of the tubing set by **1000 mL** transfer bags
Buffer Waste Bag			
Priming Waste Bag		-	
Cell Collection Bag	1 × 600 mL	1–2 × 600 mL	2 × 600 mL
Luer/Spike Interconnector	1	3	3
Plasma transfer set	-	1–2	3

Table 4.1-2: Materials required for depletion of CD4 positive cells using separation program DEPLETION 2.1.

37000 – ch15/15 (Issued: 2015-08)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement each required liter of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5 % (w/v), i.e., add 5 g HSA per liter buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

- Label one (or two) transfer bag(s) (see tables 4.1-1 and 4.1-2) as: **Cell Collection**. (Labeling should include patient identification, date and time of run, and operator identification.) Insert a Luer/Spike Interconnector into the outside port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL transfer bag as: **Cell Preparation**. (This should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL Transfer Bag as: **Plasma Waste**. (This should include patient identification, date, time of run and operator identification.)

- Label one 600 mL Transfer Bag as: **Wash Waste**. (This should include patient identification, date and time of run, operator identification.)

Important

- Note that **HSA is not a component of the CliniMACS Plus System.** Use only FDA licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.

- Store the buffer for cell preparation at +19 °C to +25 °C. Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the coupler can vary during the preparation procedure be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- All bag handling should be performed in a sterile environment (e.g. laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

III. Leukapheresis product

The following sections describe the recommended procedure for the preparation of the leukapheresis product using the Terumo Sterile Connection Device (TSCD).

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Analysis of leukapheresis product

Before starting the preparation of the leukapheresis product the following parameters should be determined:

- Total number of leukocytes,
- Percentage of CD4 positive cells
- Total number of CD4 positive cells,
- Viability.

Recheck these parameters after the labeling procedure. This is necessary because the following three sample parameters must be entered in the software during the setup of the CliniMACS ^{plus} Instument prior to loading the tubing set onto the instrument:

- Concentration of leukocytes per mL,
- Percentage of CD4 positive cells,
- Sample loading volume.

Other tests might be required depending on the intended use of the cells or individual laboratory practice.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Transfer leukapheresis product into Cell Preparation Bag

1. Record the date and the start time before beginning to prepare the leukapheresis product.

2. Determine the volume of the original leukapheresis product by estimating 1 mL of leukapheresis product as equivalent to 1 g (1 g = 1 mL).

3. Holding the leukapheresis product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the Cell Preparation Bag to the original leukapheresis product bag.

5. Open the locking forcepts to transfer the leukapheresis product material into the Cell Preparation Bag. Use a tubing stripper to clear the tubing of any remaining blood. Close the clamp.

6. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see fig. 4.1-6). Retain the original leukapheresis bag until separation and final analysis have been completed.

7. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the leukapheresis product. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS PRODUCT (should include patient identification) and retain for cell analysis. Analysis of the WBC content, CD4 enumeration and viability testing are recommended.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the actual weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with leukapheresis product. Record the weight.

Weight of leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

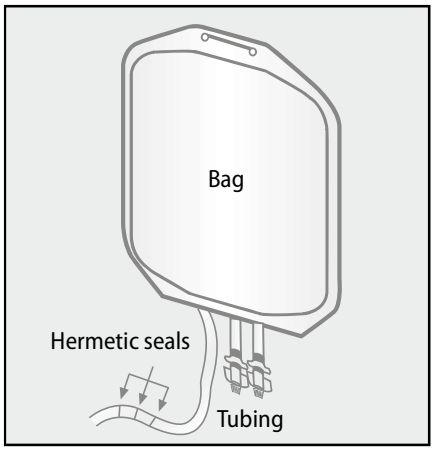


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Dilution of leukapheresis product

The leukapheresis product must be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Calculate the amount of buffer to be added using the following equation and record it.

Weight of buffer to be added (g)	=	Weight of leuka-pheresis product (g)	×	2

1. Take a plasma transfer set and ensure the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

4. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, make three hermetic seals between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the weight of the diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of diluted leukapheresis product (g)	=	Weight of filled Cell Pre paration Bag (g)	–	Weight of empty Cell Preparation Bag (g)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Volume adjustment

1. For magnetic labeling of CD4 positive cells, the optimal weight of the cell sample must be adjusted to a) 95 g (± 5 g) for the normal scale application (one vial of CliniMACS CD4 Reagent) or b) 190 g (± 5 g) for the large scale application (two vials of CliniMACS CD4 Reagent).

   Calculate the amount of supernatant to be removed to adjust the sample to 95 g (or 190 g) using the equation below:

   a) Weight of supernatant to be removed (g) = Weight of diluted leukapheresis product (g) – 95 g

   b) Weight of supernatant to be removed (g) = Weight of diluted leukapheresis product (g) – 190 g

   Record the amount of supernatant to be removed.

2. Place the empty Plasma Waste Bag on the balance and tare the balance.

Important

- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is in the open position before beginning the transfer. Release the extractor handle slowly.
- During removal of supernatant be careful not to lose cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is in the open position before beginning the transfer. Release the extractor handle slowly.
- During removal of supernatant be careful not to lose cells.

3. Open the locking forceps next to the Cell Preparation Bag. Use the balance display to monitor the weight of the Plasma Waste Bag while removing supernatant. Using the plasma extractor, remove the supernatant. Continue until sufficient amount of supernatant has been transferred into the Plasma Waste Bag, leaving a) 95 g or b) 190 g in the Cell Preparation Bag.

4. Close the locking forceps of the tubing, when the calculated weight in the Plasma Waste Bag has been reached. Record the weight of the supernatant removed.

5. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag bag.

6. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

7. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

8 Determine the total weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

9. Keep the Plasma Waste Bag until the separation and final analysis of all cells has been completed.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

IV. Magnetic labeling of the cells

The CliniMACS CD4 Reagent vial (7.5 mL) is ready to use and sufficient for one normal scale application as described below. For a large scale application, two vials of CliniMACS CD4 Reagent are required. The reagent is not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze. The reagent is to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent are printed on the reagent vial. DO NOT use the reagent after the use-by date.

Incubation with the CliniMACS® CD4 Reagent

1. Record the lot number and use-by date of the CliniMACS® CD4 Reagent(s).

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from a) one or b) two CliniMACS CD4 Reagent vials (7.5 mL each). A 10 mL syringe is sufficient to remove the contents of one vial for a normal scale application, or respectively, a 20 mL syringe is sufficient to remove the contents of two vials of reagent for a large scale application. The syringe should be equipped with a 20 gauge needle.

3. Using the injection port on the sampling site coupler, inject the entire volume of reagent into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

5. Place the Cell Preparation Bag flat on the rotator at approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Removal of excess reagent

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 500 mL of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer (i.e. add 400 g to 500 g of buffer). To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of the filled Cell Preparation Bag. Close the locking forceps next to the Cell Preparation Bag.

5. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag on the balance. Tare the balance.

14. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much supernatant as possible from the Cell Preparation Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

15. Close locking forceps and using the heat sealer, seal off the tubing to disconnect the Wash Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.

16. Keep the Wash Waste Bag until the separation and final analysis of all cells has been accomplished.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of leuka-pheresis product after the wash (g)	=	Weight of filled Cell Pre paration Bag (g)	–	Weight of empty Cell Pre paration Bag (g)

19. Adjust sample loading volume: Calculate the amount of buffer necessary to adjust the weight of the cell suspension to approximately a) 100 g (normal scale application) or b) 200 g (large scale application). For loading the labeled and washed cells on the tubing set, a maximum cell concentration of 0.4×10^9 cells per mL is recommended.

a)	Amount of buffer to be to be added (g)	=	100 g	–	Weight of leuka-pheresis product after the wash (g)
b)	Amount of buffer to be to be added (g)	=	200 g	–	Weight of leuka-pheresis product after the wash (g)

20. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

21. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

22. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

23. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

24. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

25. Resuspend the cell pellet in the Cell Preparation Bag. Avoid too intensive mixing of the cells. Ensure that all cells are resuspended.

26. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (should include patient identification) and retain for cell analysis. Analysis of the WBC content, CD4 enumeration and viability testing are recommended.

27. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the addition of buffer (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of leuka-pheresis product after buffer addition (g)	=	Weight of filled Cell Pre paration Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® CD133 Reagent (Bone Marrow)

I. General information, warnings and precautions

General information

The CliniMACS® Plus CD133 (BM) System including the CliniMACS Plus Instrument, the CliniMACS CD133 Reagent, the CliniMACS Tubing Set, and CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human CD133 positive cells from bone marrow aspirate. For peripheral blood cells as starting material, see chapter 4, STEP 1 CliniMACS CD133 Reagent.

The CD133 antigen is specifically expressed on an immature subset of CD34 positive cells. The molecule is a 5-transmembrane cell surface antigen with a molecular weight of 117 KD and is expressed on 30-60% of all CD34 positive cells including CD34 bright cells. CD133 is expressed on lineage non-committed stem and progenitor cells.

The CliniMACS Plus CD133 (BM) System uses selective CD133 monoclonal antibodies conjugated to super-paramagnetic particles. The CD133 positive cells are specifically labeled by incubation with the CliniMACS CD133 Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the enrichment in an automated continuous flow separation process.

The CliniMACS Plus CD133 (BM) System passes the antibody-labeled suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled CD133 positive target cells while non-labeled cells (non-target cells) flow through the column and are collected in the Negative Fraction Bag. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged, and the magnetic field is removed. The target cells are collected in the Cell Collection Bag.

Important

For peripheral blood cells are used as the cellular starting material, please refer to chapter 4, STEP 1 "CliniMACS CD133 Reagent" (2nd green flag).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, bone marrow aspirate, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® CD133 Reagent

Intended purpose

- The CliniMACS® CD133 Reagent is intended for the *in vitro* enrichment of human CD133 positive cells from heterogeneous hematologic cell populations in combination with the CliniMACS System only.

- The CliniMACS Plus CD133 (BM) System is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS CD133 Reagent, the CliniMACS Tubing Set LS, and CliniMACS PBS/EDTA Buffer.

Composition

Each CliniMACS CD133 Reagent vial contains 7.5 mL of a colloidal solution of iron-dextran beads conjugated to murine monoclonal CD133 antibody in PBS/EDTA buffer stabilized with Poloxamer 188 (0.03% w/v).

Side-effects

- When the enriched CD133 positive cells are infused or injected into patients, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndromes. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease could be more susceptible to iron-dextran reactions.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Precautions

- Before use in human applications, the suitability of target cells must be demonstrated regarding indication, quality and quantity.
- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively within the responsibility of the user.
- The enrichment of CD133 positive cells using the CliniMACS CD133 Reagent must be performed by trained operators only.
- Operators using the CliniMACS CD133 Reagent should have experience in the separation of cells from bone marrow or peripheral blood.
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- The CliniMACS CD133 is for *in vitro* use only. Do not inject or infuse the reagent directly into the patient. Not for parenteral application.
- The reagent is not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The reagent is for single use only (see fig. 4.1-1).
- Do not use the reagent after the use-by date printed on the vial label (see fig. 4.1-2).
- Do not use the reagent if package is damaged (see fig. 4.1-3). Use undamaged and sealed vials only.
- Store at +2 °C to +8 °C (see fig. 4.1-4). Do not freeze.

Further information

- The CliniMACS CD133 Reagent is tested for endotoxins.
- The reagent is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

Important

This protocol requires the use of components which are not part of the CliniMACS Plus CD133 System (e.g., HSA). Therefore, all materials used must be pharmaceutical grade or FDA-licensed for human use.

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

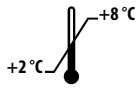


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Fig. 4.1-5: Sterilized using aseptic processing techniques.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

II. First actions required

Important

- The bone marrow aspirate contains high amounts of red blood cells. Therefore, an aspirate volume of 50 mL might lead to a labeling volume of greater than 95 mL. In this case, the labeling volume must be adjusted to 190 mL and a second CliniMACS CD133 Reagent must be used.

CliniMACS® Materials required

- **CliniMACS® CD133 Reagent, REF 172-01.**
  One or two vials are required for the application using the CliniMACS Plus CD133 (BM) System.

- **CliniMACS Tubing Set LS, REF 162-01.**
  One CliniMACS Tubing Set LS must be used for the the enrichment of CD133 positive cells from bone marrow aspirate.

- **CliniMACS PBS/EDTA Buffer, REF 700-25.**
  CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. For the preparation procedure, one liter of buffer is required. For the separation, one liter of buffer is required. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v). Only FDA-licensed HSA should be used.

Additional materials required

- **Cell Preparation Bag:**
  One 400 mL transfer tag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure.

- **Plasma Waste Bag and Wash Waste Bag:**
  Three 400 mL transfer bags, suitable for centrifugation.

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 “Additional materials required”.

- **Bone Marrow Aspirate Bag:**
  One 400 mL transfer bag in combination with a Luer/Spike Interconnector.

- **Cell Collection Bag:**
  One 150 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set.

- Pre-system filter.

- One 200 µm in-line blood filter.

- Locking forceps.

- Appropriate syringes (1 mL, 5 mL, 10 mL or 20 mL) and hypodermic 20 gauge needles.

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

- Clinical grade immunoglobulin G.

- Sample tubes.

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3: "Equipment required".

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement each required of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v), i. e. add 5 g HSA per liter buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

A master copy of a worksheet is provided with the user manual to record important information and data generated during sample handling and preparation.

- Label one 150 mL transfer bag as: **Cell Collection**. (Labeling should include patient identification, date and time of run, and operator identification.) Insert a Luer/Spike Interconnector into the outside port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight on the worksheet (C_5).

- Label one 400 mL transfer bag as: **Bone Marrow Aspirate**. (This should include patient identification, date and time of run, and operator identification.) Insert a Luer/Spike Interconnector into the outside port of the Bone Marrow Aspirate Bag.

- Label one 400 mL transfer bag as: **Cell Preparation**. (This should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight on the worksheet (A_2).

Important

- Note that **HSA is not a component of the CliniMACS Plus System**. Use only FDA-licensed HSA. Carefully read the package insert of the HSA used.

- Store the buffer for cell preparation at +19 °C to +25 °C. Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the coupler can vary during the preparation procedure be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

- Label one 400 mL transfer bag as: **Plasma Waste**. (This should include patient identification, date, time of run and operator identification.)

- Label two 400 mL transfer bag as: **Wash Waste No. 1** and **Wash Waste No. 2**. (This should include patient identification, date and time of run, operator identification.)

III. Bone marrow aspirate

The following sections describe the recommended procedure for the preparation of the bone marrow aspirate using the Terumo Sterile Connection Device (TSCD).

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Analysis of bone marrow aspirate

Before starting the preparation of the bone marrow aspirate the following parameters should be determined.

- Total number of leukocytes,
- Viability.

Important

- All bag handling should be performed in a sterile environment (e.g., laminar flow hood) using aseptic techniques. The connection of the tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Filtration of Bone Marrow Aspirate and transfer into Cell Preparation Bag

1. Record the date and the start time on the worksheet before beginning with the procedure (A_1).

2. Combine all bone marrow aspirates by transferring each aspirate into the Bone Marrow Aspirate bag through the luer lock interconnector or by using the TSCD

3. Holding the Bone Marrow Aspirate bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the filled Bone Marrow Aspirate bag to one site of the in-line blood filter, and the Cell Preparation Bag to the other site of the filter. Secure the tubing with clamps to close the fluid pathway.

5. Open the fluid pathway and hold the Bone Marrow Aspirate bag up to let the bone marrow aspirate run through the in-line blood filter into the Cell Preparation Bag. Use a tubing stripper to clear the tubing from any remaining blood. Close the clamp on the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation for further connections (see fig. 4.1-6). Retain the original Bone Marrow Aspirate bag until cell separation and final cell analysis have been completed.

7. Determine the volume of the filtered bone marrow aspirate by estimating 1 mL of cellular product as equivalent to 1 g (1 g = 1 mL).

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight on the worksheet (A_3).

9. Determine the actual weight of the bone marrow aspirate by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the Cell Preparation Bag filled with bone marrow aspirate (A_3). Record the weight on the worksheet (A_4).

10. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the bone marrow aspirate. Transfer the sample into a sample tube. Label the tube as BONE MARROW ASPIRATE (This should include patient identification.) and retain for cell analysis. Analysis of the WBC content, CD133 enumeration and viability testing are recommended.

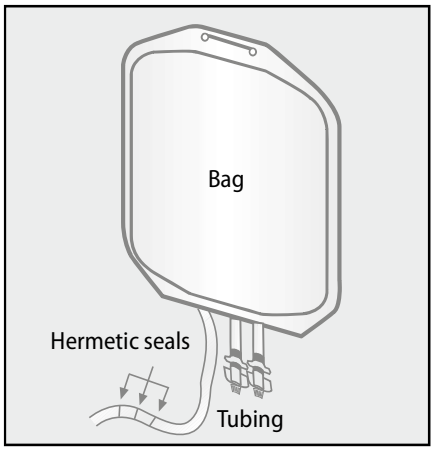


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

Worksheet equation:
$A_4 = A_3 - A_2$

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Dilution of bone marrow aspirate

The bone marrow aspirate must be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Dilute the bone marrow aspirate with buffer. Calculate the amount of buffer to be added using the following equation and record it on the worksheet (A_5).

Worksheet equation:
$A_5 = A_4 \times 4$

Weight of buffer to be added (g) (A_5)	=	Weight of bone marrow aspirate (g) (A_4)	×	4

1. Take a plasma transfer set and ensure the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

4. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer (A_5) to the Cell Preparation Bag.

5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the actual amount of buffer added on the worksheet (A_6).

6. Using the heat sealer, make three hermetic seals between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Weigh the Cell Preparation Bag and record the weight on the worksheet (A_7).

Worksheet equation:
$A_8 = A_7 - A_2$

9. Determine the total weight of diluted bone marrow aspirate by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the filled Cell Preparation Bag (A_7). Record the weight on the worksheet (A_8).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C). Record centrifugation conditions on the worksheet (A_9).

5. Remove the bag from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Volume adjustment

1. For magnetic labeling of CD133 positive cells, the optimal weight of the cell sample is 95 g (± 5 g) using one vial of CliniMACS CD133 Reagent.

   Calculate the amount of supernatant to be removed to adjust the sample to 95 g using the equation below:

   Weight of supernatant to be removed (g) = Weight of diluted bone marrow aspirate (g) – 95 g

   Worksheet equation: $A_{10} = A_8 - 95\ g$

   Record the amount of supernatant to be removed on the worksheet (A_{10}).

2. Place the empty Plasma Waste Bag on the balance and tare the balance.

3. Open the locking forceps next to the Cell Preparation Bag. Use the balance display to monitor the weight of the Plasma Waste Bag while removing the supernatant. Using the plasma extractor, carefully press out excess supernatant until the calculated "weight of supernatant to be removed" (A_{10}) has been transferred into the Plasma Waste Bag so that 95 g remain in the Cell Preparation Bag. If necessary, stop the supernatant removal before cells get transferred into the Plasma Waste Bag.

Important

- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is in the open position before beginning the transfer. Release the extractor handle slowly.
- During removal of supernatant be careful not to lose cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

4. When the appropriate amount of supernatant has been transferred, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Record the weight of the supernatant removed on the worksheet (A_{11}).

5. Using the heat sealer, seal off the tubing to disconnect the Plasma Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.

6. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight on the worksheet (A_{12}).

7. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

Worksheet equation:
$A_{13} = A_{12} - A_2$

8. Determine the weight of the bone marrow aspirate after volume adjustment by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the filled Cell Preparation Bag (A_{12}). Record the calculated weight on the worksheet (A_{13}).

9. **If the weight of the bone marrow aspirate exceeds 95 g (± 5 g), the product must be adjusted to 190 g (± 5 g) and two vials of CliniMACS CD133 Reagent are required. If not, please proceed to number 19. of the protocol.**

10. Calculate the amount of buffer to be added using the following equation and record it on the worksheet (A_{14}).

Worksheet equation:
$A_{14} = 190\ g - A_{13}$

Weight of buffer to be added (g) (A_{14}) = 190 g – Weight of diluted bone marrow aspirate (g) (A_{13})

11. Using the TSCD, connect the Buffer Bag tubing to the Cell Preparation Bag tubing. Hang the buffer bag on the plasma extractor or, alternatively, hang the buffer bag on a bag hanger.

12. Place the Cell Preparation Bag on the balance and tare the balance.

13. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the buffer bag plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer (A_{14}) to the Cell Preparation Bag.

14. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the actual amount of buffer added on the worksheet (A_{15}).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

15. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

16. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

17. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight on the worksheet (A_{16}).

18. Determine the weight of the bone marrow aspirate after the second volume adjustment (A_{16}) by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the Cell Preparation Bag. Record the calculated weight on the worksheet (A_{17}).

19. Keep the Plasma Waste Bag until the separation and final analysis of all cells has been completed.

IV. Magnetic labeling of cells

Incubation with clinical grade immunoglobulin

Disinfect the septum of the sampling site coupler of the Cell Preparation Bag. Use an appropriate sterile syringe and needle to add 1.5 mg human IgG per mL cellular product (e.g. 1.6 mL of Gamunex 10% for 95 mL cellular product). Mix the contents thoroughly. After adding the human IgG solution, the total volume should not exceeded 100 mL for a 95 mL cellular product, or 195 for a 190 mL cellular product. Incubate for 5 minutes at controlled room temperature by using a gentle rotating motion (e.g. 25 rpm on orbital rotator).

Important

- Note that **human IgG is not a component of the CliniMACS Plus System.** Use only pharmaceutical grade human IgG with appropriate approvals. Carefully read the package insert of the human IgG used; in particular the section regarding hypersensitivity reactions and the risk of infection that human IgG as a blood derived product brings to all patients. All risks arising from these materials must be evaluated by the user.

Incubation with the CliniMACS® CD133 Reagent

The CliniMACS® CD133 Reagent vials (7.5 mL each) are ready to use and sufficient for one application as described below. The reagents are not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze. The reagent is to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent are printed on the vial. DO NOT use the reagent after the use-by date.

1. Record the lot number (B_1) and use-by date (B_2) of the reagent(s) on the worksheet.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from the reagent vial (7.5 mL). A 10 mL syringe is

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

sufficient to remove the contents of one reagent vial or a 20 mL syringe is sufficient to remove the contents of two reagent vials, respectively. The syringe should be equipped with a 20 gauge needle.

3. **If the labeling volume of the bone marrow aspirate was adjusted to 190 g (±5 g), a second CliniMACS CD133 Reagent vial must be added.**

4. Using the injection port on the sampling site coupler, inject the entire volume of reagent into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation.

5. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time on the worksheet (B_3).

6. Place the Cell Preparation Bag flat on the orbital rotator at approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 15 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time on the worksheet (B_4).

Removal of excess reagent: Wash No. 1 and Wash No. 2

Wash No. 1

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least one liter of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and add 300 g buffer. To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of buffer transferred into the Cell Preparation Bag on the worksheet (B_5).

5. Close the locking forceps next to the Cell Preparation Bag. Seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

7. Using the TSCD, connect the empty Wash Waste Bag No. 1 to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag No. 1 securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g for 15 min with moderate brake at room temperature (+19 °C to +25 °C). Record the centrifugation conditions on the worksheet (B_6).

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag No. 1 on the balance. Tare the balance.

14. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much excess supernatant as possible from the Cell Preparation Bag. Record the amount of removed supernatant on the worksheet (B_7).

15. Close the locking forceps and use the heat sealer to seal off the tubing to disconnect the Wash Waste Bag No. 1 leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.

16. Keep the Wash Waste Bag No. 1 until the separation and final analysis of all cells has been completed.

Wash No. 2

17. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

18. Place the Cell Preparation Bag on the balance. Tare the balance.

19. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and add 300 g buffer. To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of buffer transferred into the Cell Preparation Bag on the worksheet (B_8).

Important

During the removal of supernatant, be careful not to lose cells.

20. Close the locking forceps next to the Cell Preparation Bag. Seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

21. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

22. Using the TSCD, connect the empty Wash Waste Bag No. 2 to the Cell Preparation Bag.

23. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag No. 2 securely to the centrifuge bucket.

24. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

25. Centrifuge at 300×g for 15 min with moderate brake at room temperature (+19 °C to +25 °C). Record the centrifugation conditions on the worksheet (B_9)

26. Taking care not to disturb the cell pellet, remove the bags from the centrifuge. Carefully hang the Cell Preparation Bag on the plasma extractor.

27. Place the Wash Waste No. 2 Bag on the balance. Tare the balance.

28. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much excess supernatant as possible from the Cell Preparation Bag. Record the amount of removed supernatant on the worksheet (B_{10}).

29. Close the slide clamp and use the heat sealer to seal off the tubing on the Cell Preparation Bag.

30. Keep the Wash Waste Bag No. 2 until the final separation and analysis of the CD133 positive cells has been accomplished.

31. Resuspend the cell pellet in the Cell Preparation Bag. Avoid too intensive mixing of the cells. Ensure that all cells are resuspended.

32. Weigh the Cell Preparation Bag and record the weight on the worksheet (B_{11}).

Worksheet equation:
$B_{12} = B_{11} - A_2$

33. Determine the weight of the cellular product by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the Cell Preparation Bag after the second wash (B_{11}). Record the weight on the worksheet (B_{12}).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

34. If the determined weight ranges between 100 g and 275 g, proceed to number 41. If the volume is less than 100 g, calculate the amount of buffer necessary to adjust the weight of the cell suspension to approximately 100 g (B_{13}). The weight must not exceed 275 g.

    Worksheet equation:
    $B_{13} = 100\ g - B_{12}$

35. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

36. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

37. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer (B_{13}) to the Cell Preparation Bag.

38. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next of the Cell Preparation Bag.

39. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

40. Weigh the Cell Preparation Bag and record the weight on the worksheet (B_{14}). Determine the weight of the cellular product (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the Cell Preparation Bag after buffer addition (B_{14}). Record the weight on the worksheet (B_{15}).

    Worksheet equation:
    $B_{15} = B_{14} - A_2$

41. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (This should include patient identification.) and retain for cell analysis. WBC content, CD133 enumeration and viability testing are recommended.

Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Worksheet for preparation and enrichment of CD133 positive cells from bone marrow aspirate

Patient Data _____

_____

Operator ID _____

Filtration of Bone Marrow Aspirate and Transfer into Cell Preparation Bag

Starting: Date _____ Time _____ (A_1)

Weight of empty Cell Preparation Bag
(with locking forceps and wsampling site coupler) _____ g (A_2)

Weight of Cell Preparation Bag
filled with bone marrow aspirate _____ g (A_3)

Weight of bone marrow aspirate (difference
between filled and empty Cell Preparation Bag)
$A_4 = A_3 - A_2$ _____ g (A_4)

Remove sample: BONE MARROWASPIRATE ☐ (if yes ✓)

Dilution of Bone Marrow Aspirate

The bone marrow aspirate should be diluted with CliniMACS PBS/EDTA Buffer and centrifuged before magnetic labeling. Calculate the amount of buffer to be added using the equation below.

Weight of buffer to be added	=	Weight of bone marrow aspirate	×	4

Weight of buffer to be added
$A_5 = A_4 \times 4$ _____ g (A_5)

Actual amount of buffer added _____ g (A_6)

Weight of filled Cell Preparation
Bag after dilution _____ g (A_7)

Weight of diluted bone marrow aspirate
$A_8 = A_7 - A_2$ _____ g (A_8)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Centrifugation of Bone Marrow Aspirate

Time
Spin Speed: Acceleration: Brake:
g Force:

Volume Adjustment of Bone Marrow Aspirate

The optimal weight of the bone marrow aspirate is 95 g (± 5 g) for magnetic labeling of the CD133 positive cells using one vial of CliniMACS CD133 Reagent. If the sample weight exceeds the optimal weight, two vials of CliniMACS CD133 Reagent have to be used. Therefore, the labeling weight has to be adjusted to 190 g (± 5 g).

Calculate the amount of supernatant to be removed to give a final weight of 95 g (± 5 g). Use equation below.

Amount of supernatant to be removed	=	Total weight of diluted bone marrow aspirate (g)	–	95 g

Amount of supernatant to be removed
$A_{10} = A_8 - 95$ g _____ g (A_{10})

Actual amount of supernatant removed _____ g (A_{11})

Weight of filled Cell Preparation Bag after volume adjustment _____ g (A_{12})

Weight of bone marrow aspirate after volume adjustment
$A_{13} = A_{12} - A_2$ _____ g (A_{13})

Adjustment of bone marrow aspirate if the weight exceeds 95 mL (± 5g)

Calculate the amount of buffer to be added if the final volume exceeds 95 mL (± 5 mL).

Weight of buffer to be added	=	190 g	–	Weight of bone marrow aspirate (g)

Amount of buffer to be added (g)
$A_{14} = 190 - A_{13}$ _____ g (A_{14})

Actual amount of buffer added _____ g (A_{15})

Total weight of Cell Preparation Bag after addition of buffer _____ g (A_{16})

Weight of total bone marrow aspirate after addition of buffer
$A_{17} = A_{16} - A_2$ _____ g (A_{17})

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Incubation with clinical grade immunoglobulin

Add IgG (1.5 mg/mL), incubate on orbital rotator (25 rpm) for 5 min at RT. After adding the human IgG solution, the total volume should not exceeded 100 mL for a 95 mL cellular product, or 195 mL for a 190 mL cellular product.

Magnetic labeling of CD133 positive cells

Lot number of CD133 Reagent _____ (B_1)

Second CD133 Reagent _____ (B_1)
(if labeling volume exceeds 95 mL)

Use-by date of CD133 Reagent _____ (B_2)

Second CD133 Reagent _____ (B_2)

Incubation

Incubation start time _____ (B_3)

Incubation stop time _____ (B_4)

Wash No. 1

Amount of buffer transferred
into the Cell Preparation Bag _____ g (B_5)

Centrifugation conditions
Time:
g Force:
Temperature:
(B_6)

Amount of removed supernatant _____ g (B_7)

Wash no. 2

Amount of buffer transferred
into the Cell Preparation Bag _____ g (B_8)

Centrifugation conditions
Time:
g Force:
Temperature:
(B_9)

Amount of removed supernatant _____ g (B_{10})

Weight of filled Cell Preparation
Bag after washing procedure _____ g (B_{11})

Weight of bone marrow aspirate
after washing procedure
$B_{12} = B_{11} - A_2$ _____ g (B_{12})

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Final sample preparation

The weight of the final sample for loading on the CliniMACS Plus Instrument must range between 100 g and 275 g. If the determined weight of the cellular product (B_{12}) is less than 100 g, adjust the volume of the bone marrow aspirate to 100 mL. Calculate the amount of buffer to be added:

Amount of buffer to added
$B_{13} = 100\ g - B_{12}$ (normal scale) _____ g (B_{13})

Weight of Cell Preparation Bag
after buffer addition _____g (B_{14})

Weight of bone marrow aspirate
after buffer addition (sample loading volume)
$B_{15} = B_{14} - A_2$ _____ g (B_{15})

Remove sample: ☐ (if yes ✓)
(ORIGINAL)

CliniMACS Separation (Run)

Date, time started run: Date _____ Time _____ (C_1)

Lot number of tubing set _____ (C_2)

Use-by date of tubing set _____ (C_3)

Calculate the amount of the target cell fraction

Weight of filled Cell Collection Bag
after the run has been finished _____ g (C_4)

Weight of empty Cell Collection Bag
(with locking forceps) _____ g (C_5)

Weight of target cell fraction
$C_6 = C_4 - C_5$ _____ g (C_6)

Process code _____ (C_7)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Analysis data

This list is an example of analysis data acquired. Further analysis of additional parameters should be included based on the intended use of the selected cells.

Bone Marrow Aspirate

Sample taken before preparation procedure: BONE MARROW ASPIRATE

Total number of leukocytes _____

Viability _____

Total number of CD133 positive cells _____

Percentage of CD133 positive cells _____

Bone Marrow Aspirate

Sample taken after magnetic labeling: ORIGINAL

Total number of leukocytes _____

Viability _____

Total number of CD133 positive cells _____

Positive fraction of CD133 positive cells

Content of Cell Collection Bag

Total number of leukocytes _____

Viability _____

Total number of CD133 positive cells _____

Purity of CD133 positive cells _____

Recovery of CD133 positive cells _____

Negative fraction

Content of Negative Fraction Bag

Total number of leukocytes _____

Viability _____

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® TCRα/β-Biotin

I. General information, warnings and precautions

General information

The CliniMACS® Plus TCRα/β-Biotin System including the CliniMACS ^{plus} Instrument, the CliniMACS TCRα/β-Biotin, the CliniMACS Anti-Biotin Reagent, the CliniMACS Depletion Tubing Set, and CliniMACS PBS/EDTA Buffer, is intended for the *in vitro* depletion of human TCRα/β positive cells from heterogeneous hematologic cell populations.

The TCRα/β is the T cell receptor heterodimer composed of two transmembrane glycoprotein chains, α and β. Both chains are members of the Ig superfamily and consist of a constant and a polymorphic variable region. The variable region of the TCRα/β receptor is involved in recognition of antigenic peptides presented by the MHC complex of antigen presenting cells. The TCRα/β is expressed on the majority of peripheral blood T cells.

The CliniMACS Plus TCRα/β-Biotin System uses murine monoclonal antibodies specific for the TCRα/β antigen conjugated to biotin in combination with the CliniMACS Anti-Biotin Reagent. TCRα/β positive cells are separated in an automated separation system provided by the CliniMACS ^{plus} Instrument and the CliniMACS Depletion Tubing Set and CliniMACS PBS/EDTA Buffer.

The TCRα/β positive cells are labeled for separation by incubation with the CliniMACS TCRα/β-Biotin. After unbound conjugate is removed from the suspension, the cells are magnetically labeled with the CliniMACS Anti-Biotin Reagent. After excess reagent is removed from the suspension, the cells are ready for the automated separation process.

The CliniMACS Plus TCRα/β-Biotin System passes the antibody-labeled cell suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled TCRα/β positive cells (non-target cells), while the unlabeled cells (target cells) flow through the column. Several automated washing steps are performed, collecting the TCRα/β negative target cells in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, the cellular product, the CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® TCRα/β-Biotin

Intended purpose

- The CliniMACS® TCRα/β-Biotin is intended for the *in vitro* depletion of human TCRα/β positive cells from heterogeneous hematologic cell populations in combination with the CliniMACS System only.

- The CliniMACS Plus TCRα/β-Biotin System is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS TCRα/β-Biotin, the CliniMACS Anti-Biotin Reagent, the CliniMACS Depletion Tubing Set, and CliniMACS PBS/EDTA Buffer.

Important

Please refer also to the information regarding the CliniMACS Anti-Biotin Reagent on page 2 of STEP1: "CliniMACS® Anti-Biotin Reagent".

Composition

Each vial of CliniMACS TCRα/β-Biotin contains 7.5 mL of a solution of murine monoclonal TCRα/β antibody conjugated to biotin in PBS/EDTA buffer stabilized with Poloxamer 188 (0.03% w/v).

Side-effects

- When the TCRα/β depleted cell fraction is infused into a patient, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndromes. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease could be more susceptible to iron-dextran.

Important

This protocol demands the use of components which are not part of the CliniMACS TCRα/β-Biotin System (e.g., HSA). Therefore, all materials used must be pharmaceutical grade or FDA-licensed for human use.

Precautions

- Before use in human applications, the suitability of the target cells must be demonstrated regarding indication, quality and quantity.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively within the responsibility of the user.
- The depletion of TCRα/β positive cells using the CliniMACS TCRα/β-Biotin must be performed by trained operators only.
- Operators using the CliniMACS TCRα/β-Biotin must have experience in the separation of cells from peripheral blood.
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- The TCRα/β positive cells (non-target cell fraction) are not intended for therapeutic use and may not be reinfused into patients.
- Do not inject or infuse the conjugate directly into the patient. For *in vitro* use only. Not for parenteral application.
- The CliniMACS TCRα/β-Biotin in combination with the CliniMACS Anti-Biotin Reagent is not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The reagent is for single use only (see fig. 4.1-1).
- Do not use after the use-by date, printed on the vial label (see fig. 4.1-2).
- Do not use the if package is damaged (see fig. 4.1-4). Use undamaged and sealed vials only.
- Store at +2 °C to +8 °C (see fig. 4.1-3). Do not freeze.

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

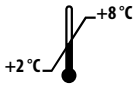


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Further information

- The CliniMACS TCRα/β-Biotin is tested for endotoxins.
- The reagent is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

Fig. 4.1-5: Sterilized using aseptic processing techniques.

II. First actions required

CliniMACS® Materials required

CliniMACS® TCRα/β-Biotin is sold as a kit. Each kit contains the following reagents:

- **CliniMACS TCRα/β-Biotin, REF 701-48.**
  One vial is required for the application using the CliniMACS Plus TCRα/β-Biotin System.

- **CliniMACS Anti-Biotin Reagent, REF 173-01.**
  Two vials are required for the application using the CliniMACS Plus TCRα/β-Biotin System.

- **CliniMACS Depletion Tubing Set, REF 261-01.**

- **CliniMACS PBS/EDTA Buffer, REF 700-25.**
  CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. For the preparation procedure, up to three liters of buffer are required. For the separation, one liter of buffer is required. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Note

The application capacity for the depletion of TCRα/β positive cells using the CliniMACS TCRα/β-Biotin System amounts to 24×10^9 TCRα/β positive cells out of a total cell number not exceeding 60×10^9 (WBC).

Additional materials required

- **Cell Preparation Bag:**
  One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets for the cell preparation procedure.

- **Plasma Waste Bag and Wash Waste Bags:**
  Four 600 mL (or 1000 mL) transfer bags, suitable for centrifugation.

- **Cell Collection Bag:**
  One 600 mL Cell Collection Bag is already assembled to the CliniMACS Depletion Tubing Set.

- Pre-system filter.

- Locking forceps or side clamps.

- Appropriate syringes (5 mL, 10 mL, 20 mL, 50 mL) and hypodermic 20 gauge needles.

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).

- Sample tubes.

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 “Additional materials required”.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3: "Equipment required".

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement each required liter of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v), i.e., add 5 g HSA per liter buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

- One 600 mL transfer bag is already assembled und labeled as: **Cell Collection**. (Labeling should include patient identification, date and time of run, and operator identification.) Weigh the empty Cell Collection Bag and record the weight.

- Label one 600 mL transfer bag as: **Cell Preparation**. (This should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL (or 1000 mL) transfer bag as: **Plasma Waste**. (this should include patient identification, date, time of run, and operator identification.)

- Label three 600 mL (or 1000 mL) transfer bags as: **Wash Waste No. 1, Wash Waste No. 2**, and **Wash Waste No. 3**. (This should include patient identification, date, time of run, and operator identification.)

Important

- Note that **HSA is not a component of the CliniMACS Plus System**. Use only FDA-licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.

- Store the buffer for cell preparation at +19 °C to +25 °C. Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the coupler can vary during the preparation procedure be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- All bag handling should be performed in a sterile environment (e.g., laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.
- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

III. Leukapheresis product

The following sections describe the recommended procedure for preparing the leukapheresis product using the Terumo Sterile Connection Device (TSCD). All bag handling should be performed in a sterile environment (e.g. laminar flow hood) using aseptic techniques.

- The operator has to be familiar with the operation and use of the TSCD.
- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Analysis of leukapheresis product

Before starting the preparation of the leukapheresis product, the following parameters should be determined:

- Volume and cell concentration to determine the total number of leukocytes,
- Percentage of TCRα/β positive cells,
- Total number of TCRα/β positive cells,
- Viability.

Determine the percentage/total number of all TCRα/β-labeled cells (including unspecific TCRα/β binding, not only real TCRα/β positive cells). Consider this number for the application specifications and for sample parameter input during the setup of the CliniMACS ^{plus} Instrument (see STEP 2).

Other tests might be required depending on the intended use of the cells or individual laboratory practice. Record all data.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Transfer leukapheresis product into Cell Preparation Bag

1. Record the date and the start time before beginning to prepare the leukapheresis product.

2. Determine the volume of the original leukapheresis product by estimating 1 mL of leukapheresis product as equivalent to 1 g (1 g = 1 mL).

3. Holding the leukapheresis product bag with both hands, mix the content thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the Cell Preparation Bag to the original leukapheresis product bag.

5. Open the locking forceps to transfer the leukapheresis product material into the Cell Preparation Bag. Use a tubing stripper to clear the tubing of any remaining blood. Close the locking forceps.

6. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see fig. 4.1-6). Retain the original leukapheresis bag until cell separation and analysis have been completed.

7. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the leukapheresis product. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS PRODUCT (should include patient identification) and retain for cell analysis.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the actual weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with leukapheresis product. Record the weight.

Weight of leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

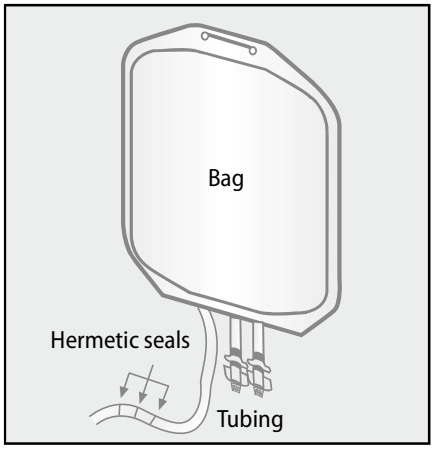


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Dilution of leukapheresis product

The leukapheresis product must be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Dilute the leukapheresis product to a total volume of three times the volume of the leukapheresis product, but not exceeding a total volume of 600 mL. Calculate the amount of buffer to be added using the following equation and record it.

Weight of buffer to be added (g)	=	Weight of leukapheresis product (g)	×	2

1. Take a plasma transfer set and ensure that the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.

2. Using the TSCD, connect the buffer bag tubing to the Cell Preparation Bag tubing. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

4. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, make three hermetic seals between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the total weight of leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of diluted leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Volume adjustment

1. For labeling of TCRα/β positive cells, the optimal weight of the cell sample is 95 g (± 5 g).

   Remove supernatant completely, taking care not to resuspend the cell pellet during removal of supernatant. When using the plasma extractor, maintain constant control of the extractor release handle and ensure the locking forceps next to the Cell Preparation Bag are in the open position before beginning the transfer of fluid. Release the extractor handle slowly being careful not to lose cells. Close the locking forceps, when the supernatant has been removed. Seal off the tubing to disconnect the Plasma Waste Bag. Resuspend the pellet carefully.

   Place the Cell Preparation Bag with the cell pellet on the balance. Adjust the weight of the Cell Preparation Bag by adding buffer. Calculate the weight of the Cell Preparation Bag filled with diluted leukapheresis product using the equation below:

Target weight of of Cell Preparation Bag filled with diluted leukapheresis product (g)	=	95 g	+	Weight of empty Cell Preparation Bag (g)

2. Insert the spike of a transfer set to a port of a buffer bag containing one liter of buffer. Confirm the clamp on the plasma transfer set is closed.

3. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

Important

- When using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps on the Cell Preparation Bag is in the open position before beginning the transfer. Release the extractor handle slowly being careful not to lose cells.
- During removal of supernatant be careful not to lose cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

4. Tare the balance. Place the Cell Preparation Bag on the balance.

5. Open the locking forceps next to Cell Preparation Bag. Then open the clamp on the plasma transfer set and fill the Cell Preparation Bag with buffer until the calculated "Target weight of Cell Preparation Bag filled with diluted leukapheresis product (g)" is reached. To stop the liquid flow, close the clamp on the plasma transfer set. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

8. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

9. Determine the total weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

10. Keep the Plasma Waste Bag until the separation and final analysis of cells has been completed.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

IV. Labeling of the cells with the CliniMACS® TCRα/β-Biotin

One vial (7.5 mL) CliniMACS® TCRα/β-Biotin is ready to use and sufficient for one application as described below. The conjugate is not for parenteral administration.

Store the conjugate at +2 °C to +8 °C. DO NOT freeze. The conjugate is to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the conjugate are printed on the vial. DO NOT use the conjugate after the use-by date.

Incubation with CliniMACS® TCRα/β-Biotin

1. Record the lot number and use-by date of the CliniMACS® TCRα/β-Biotin vial.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from one CliniMACS TCRα/β-Biotin vial (7.5 mL). A 10 mL syringe is sufficient to remove the contents of one vial. The syringe should be equipped with a 20 gauge needle.

3. Using the injection port on the sampling site coupler, inject the entire volume of conjugate into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

5. Place the Cell Preparation Bag flat on the orbital rotator, set to a speed of approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Removal of excess conjugate

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 500 mL of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag tubing to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer. To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of the filled Cell Preparation Bag.

5. Close the locking forceps next to the Cell Preparation Bag. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag No. 1 to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag No. 1 on the balance. Tare the balance.

14. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much excess supernatant as possible from the Cell Preparation Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

15. Close the locking forceps and using the heat sealer, seal off the tubing to disconnect the Wash Waste Bag No. 1 leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.

16. Keep the Wash Waste Bag until the separation and final analysis of cells has been accomplished.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. **Repeat "washing procedure" (step 1 to 17) using the Wash Waste Bag No. 2, and then continue with step 19.**

19. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the second wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag after the washes. Record the weight.

Weight of leukapheresis product after the second wash (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

20. Calculate the weigt of buffer necessary to adjust the weight of the cell suspension to approximately 190 g, for magnetic labeling of the TCRα/β-Biotin labeled cells with the CliniMACS Anti-Biotin Reagent.

Weightof buffer to be added (g)	=	190 g	–	Weight of leukapheresis product after the second wash (g)

21. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

22. Place the Cell Preparation Bag on the balance and tare the balance.

23. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

24. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

25. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

Important

Repeat "washing procedure" (step 1 to 17) using the Wash Waste Bag No. 2.

V. Magnetic labeling of the cells with the CliniMACS® Anti-Biotin Reagent

The CliniMACS® Anti-Biotin Reagent vials (7.5 mL each) are ready to use and sufficient for one application as described below. The reagents are not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze. The reagent is to be used cold and directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent is printed on the vial. DO NOT use the reagent after the use-by date.

Incubation with CliniMACS® Anti-Biotin Reagent

1. Record the lot number and use-by date of the CliniMACS® Anti-Biotin Reagent vials.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from each of the two CliniMACS Anti-Biotin Reagent vials (7.5 mL each). A 20 mL syringe is sufficient to take the contents of two vials. The syringe should be equipped with a 20 gauge needle.

3. Using the injection port on the sampling site coupler, inject the entire volume of both reagent vials into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

5. Place the Cell Preparation Bag flat on the orbital rotator, set to a speed of approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Removal of excess reagent

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 500 mL of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag tubing to the Cell Preparation Bag tubing. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer. To stop the liquid flow, close the clamps on the plasma transfer set. Close the locking forceps next to the Cell Preparation Bag. Record the weight of the filled Cell Preparation Bag.

5. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag No. 3 to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag No. 3 on the balance. Tare the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

14. Open the locking forceps next to the Cell Preparation Bag tubing. Using the plasma extractor, remove as much supernatant as possible from the Cell Preparation Bag.

15. Close the locking forceps and using the heat sealer, seal off the tubing to disconnect the Wash Waste Bag No. 3 leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.

16. Keep the Wash Waste Bag until the separation and final analysis of all cells has been completed.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of leuka-pheresis product after the wash (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Note

For loading the labeled and washed cells on the CliniMACS Depletion Tubing Set, a maximum cell concentration of 0.4×10^9 cells per mL is recommended .

19. Adjust sample loading volume: Calculate the amount of buffer necessary to adjust the weight of the cell suspension to approximately 150 g. For loading the labeled and washed cells on the tubing set, a maximum cell concentration of 0.4×10^9 cells per mL is recommended.

Amount of buffer to be to be added (g)	=	150 g	–	Weight of leuka-pheresis product after the wash (g)

20. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

21. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

22. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

23. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

24. Using the heat sealer, make three hermetic seals between both clamps. Disconnect the buffer bag.

25. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a sample of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (should include patient identification) and retain for cell analysis.

26. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag after buffer addition. Record the weight.

Weight of leukapheresis product after addition of buffer (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Proceed to STEP 2

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® CD45RA Reagent

I. General information, warnings and precautions

General information

The CliniMACS® Plus CD45RA System including the CliniMACS plus Instrument, the CliniMACS CD45RA Reagent, the CliniMACS Depletion Tubing Set, and CliniMACS PBS/EDTA Buffer is intended for the *in vitro* depletion of human CD45RA positive cells from heterogeneous hematologic cell populations.

The CD45RA antigen is expressed on naive CD4 and CD8 positive T cells, as well as on subsets of B cells, NK cells, and monocytes. The overall frequency of CD45RA positive cells including CD45RA dim cells in PBMC is 55-75 %. The CD45RA antibody recognizes the 220 kDa isoform of the leukocyte common antigen (LCA), a transmembrane tyrosine phosphatase.

The CliniMACS CD45RA Reagent consists of selective CD45RA monoclonal antibodies conjugated to super-paramagnetic particles. The CD45RA positive cells are specifically labeled by incubation with the CliniMACS CD45RA Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the depletion in an automated continuous flow separation process.

The CliniMACS Plus CD45RA System passes the antibody-labeled cell suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled CD45RA positive cells (non-target cells), while the unlabeled cells (target cells) flow through the column. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged, and the magnetic field is removed. The target cells are collected in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, the cellular starting product, the CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® CD45RA Reagent

Intended purpose

- The CliniMACS® CD45RA Reagent is intended for the *in vitro* depletion of human CD45RA positive cells from heterogeneous hematologic cell populations in combination with the CliniMACS System only.

- The CliniMACS Plus CD45RA System is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS Depletion Tubing Set, and CliniMACS PBS/EDTA Buffer.

Composition

Each vial of CliniMACS CD45RA Reagent contains 7.5 mL of a colloidal solution of iron-dextran beads conjugated to murine monoclonal CD45RA antibody in PBS/EDTA buffer stabilized with Poloxamer 188 (0.03% w/v).

Side-effects

- When the CD45RA depleted cell fraction is infused or injected into patients, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndromes. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease could be more susceptible to iron-dextran reactions.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Precautions

- Before use in human applications, the suitability of the CD45RA-depleted target cell fraction must be demonstrated regarding indication, quality and quantity.
- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively the responsibility of the user.
- The depletion of CD45RA positive cells using the CliniMACS CD45RA Reagent must be performed by trained operators only.
- Operators using the CliniMACS CD45RA Reagent should have experience in the separation of cells from bone marrow or peripheral blood.
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- The CliniMACS CD45RA Reagent is for *in vitro* use only. Do not inject or infuse the reagent directly into the patient. Not for parenteral application.
- The reagent is not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The reagent is for single use only (see fig. 4.1-1).
- Do not use the reagent after the use-by date printed on the vial label (see fig. 4.1-2).
- Store at +2 °C to +8 °C (see fig. 4.1-3). Do not freeze.
- Do not use the reagent if package is damaged (see fig. 4.1-4). Use undamaged and sealed vials only.

Further information

- The CliniMACS CD45RA Reagent is tested for endotoxins.
- The reagent is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

Important

This protocol requires the use of components which are not part of the CliniMACS Plus CD45RA System (e.g., HSA). Therefore, all materials used must be pharmaceutical grad or FDA-licensed for human use.

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

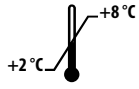


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Fig. 4.1-5: Sterilized using aseptic processing techniques.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

II. First actions required

CliniMACS® Materials required

Note

The application capacity for the depletion of CD45RA positive cells using the CliniMACS Plus CD45RA System is 20×10^9 CD45RA positive cells out of a total cell number not exceeding 50×10^9 (WBC).

- **CliniMACS® CD45RA Reagent, REF 701-46.**
  One vial is required for the application using the CliniMACS Plus CD45RA System.

- **CliniMACS Depletion Tubing Set, REF 261-01.**
  One CliniMACS Tubing Set must be used for the depletion of CD45RA positive cells.

- **CliniMACS PBS/EDTA Buffer, REF 700-25.**
  CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. For the preparation procedure, two liters of buffer are required. For the separation, one liter of buffer is required. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v). Use only FDA-licensed HSA.

Additional materials required

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 "Additional materials required".

- **Cell Preparation Bag:**
  One 600 mL transfer bag suitable for centrifugation as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure.

- **Plasma Waste and Wash Waste Bag:**
  Two 600 mL transfer bags suitable for centrifugation.

- **Cell Collection Bag:**
  One 600 mL Cell Collection Bag is already assembled to the CliniMACS Depletion Tubing Set.

- Pre-system filter.

- Locking forceps or slide clamps.

- Appropriate syringes (1 mL, 10 mL or 20 mL) and hypodermic 20 gauge needles.

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).

- Sample tubes.

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3: "Equipment required".

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement each required liter of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5 % (w/v), i.e. add 5 g HSA per liter Buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

A master copy of a worksheet is provided with the user manual to record important information and data generated during sample handling and preparation.

- Label one 600 mL transfer bag as: **Cell Preparation** (this should include patient identification, date and time of run, and operator identification). Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight on the worksheet (A_1).

- Label one 600 mL Transfer Bag as: **Plasma Waste** (this should include patient identification, date, time of run and operator identification).

- Label one 600 mL Transfer Bag as: **Wash Waste** (this should include patient identification, date and time of run, operator identification).

Important

- Note that **HSA is not a component of the CliniMACS Plus System**. Use only FDA-licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.

- Store the buffer for cell preparation at +19 °C to +25 °C. Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since that the length of the coupler can vary during the preparation procedure be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- All bag handling should be performed in a sterile environment (e.g. laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

- An alternative starting cell source can be the negative cell fraction of a CliniMACS Plus CD34 Separation.

- The use of the CliniMACS CD45RA Reagent is only recommended for cellular starting products containing CD45RA positive cells within the normal frequency range (up to 80%). Higher frequencies have been observed in a few donors where CD45RA is expressed by almost all PBMC due to a rare mutation within the CD45 gene (1-13% of healthy donors depending on the ethnic group). In such cases, the depletion efficiency and/or recovery may be less than expected.

III. Leukapheresis product

The following sections describe the recommended procedure for preparing the leukapheresis product using the Terumo Sterile Connection Device (TSCD). All bag handling should be performed in a sterile environment (e.g. laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Analysis of leukapheresis product

Before starting the preparation of the leukapheresis product the following parameters should be determined:

- Total number of leukocytes,
- Percentage of CD45RA positive cells,
- Total number of CD45RA positive cells,
- Viability.

Recheck these parameters after the labeling procedure. This is necessary because the following three sample parameters must be entered in the software during the setup of the CliniMACS ^{plus} Instument prior to loading the tubing set onto the instrument:

- Concentration of leukocytes per mL,
- Percentage of CD45RA positive cells,
- Sample loading volume.

Other tests might be required depending on the intended use of the cells or individual laboratory practice.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Transfer leukapheresis product into Cell Preparation Bag

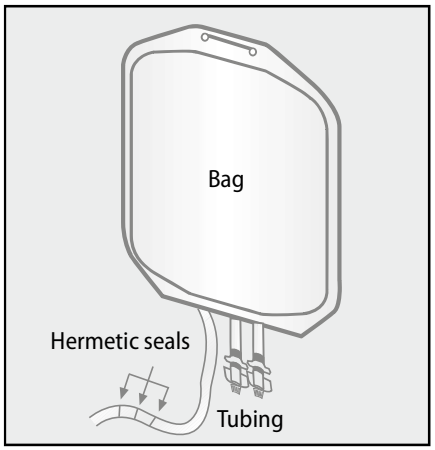


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

1. Record the date and the start time before preparing the leukapheresis product.

2. Determine the volume of the original leukapheresis product by estimating 1 mL of leukapheresis product as equivalent to 1 g (1 g = 1 mL).

3. Holding the leukapheresis product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the Cell Preparation Bag to the original leukapheresis product bag.

5. Open the locking forceps to transfer the leukapheresis product material into the Cell Preparation Bag. Use a tubing stripper to clear the tubing of any remaining blood. Close the locking forceps.

6. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see fig. 4.1-6). Retain the original leukapheresis bag until cell selection and analysis have been completed.

7. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the leukapheresis product. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS PRODUCT (should include patient identification) and retain for cell analysis. Analysis of the WBC content, CD45RA enumeration and viability testing are recommended.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag (A_2) on the worksheet.

9. Determine the actual weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag (A_1) from the weight of the Cell Preparation Bag filled with leukapheresis product (A_2). Record the weight on the worksheet (A_3).

Weight of leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Worksheet equation: $A_3 = A_2 - A_1$

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Note

If the negative fraction of a CliniMACS Plus CD34 Separation is used as alternative starting cell source, the dilution can be skipped. Please continue with the centrifugation step and centrifuge the cells at 300×g (without brake) for 15 minutes at room temperature (+19 °C to +25 °C).

Dilution of leukapheresis product

The leukapheresis product must be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Dilute the leukapheresis product 1:3 with buffer. Calculate the amount of buffer to be added using the following equation and record it on the worksheet (A_4). Consider that this 1:3 dilution requires an apheresis product volume not higher than 200 mL.

Worksheet equation:
$A_4 = A_3 \times 2w$

Weight of buffer to be added (g)	=	Weight of leukapheresis product (g)	×	2

1. Take a plasma transfer set and ensure the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.

2. Using the TSCD, connect the buffer bag tubing to the Cell Preparation Bag tubing. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

4. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag (A_4).

5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the weight of the filled Cell Preparation Bag (A_5) on the worksheet.

6. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Determine the total weight of diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag (A_1) from the weight of the filled Cell Preparation Bag (A_5). Record the weight on the worksheet (A_6).

Worksheet equation:
$A_6 = A_5 - A_1$

Weight of diluted leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C). If the negative fraction of a CliniMACS Plus CD34 Separation is used as starting cell source centrifuge the cells at 300xg for 15 min (without brake) at room temperature (+19 °C to +25 °C).

5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Important

All bags, including those used in sample preparation, should be preserved until final analysis of the all cells has been completed and sucessful separation of the target cells has been confirmed.

Volume adjustment

1. For magnetic labeling of CD45RA positive cells, the optimal weight of the cell sample is 95 g (± 5 g).

   Remove supernatant completely, taking care not to resuspend the cell pellet during removal of supernatant. When the supernatant has been removed, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag. Resuspend the pellet carefully.

   Tare the balance and weigh the filled Cell Preparation Bag. Record the weight on the worksheet (A_7).

   Calculate the weight of the leukapheresis product after removal of supernatant by subtracting the weight of the empty Cell Preparation Bag (A_1) from the weight of the filled Cell Preparation Bag (A_7). Record the calculated weight on the worksheet (A_8).

Weight of leukapheresis product after removal of supernatant (g	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Important

- When using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps on the Cell Preparation Bag is in the open position before beginning the transfer. Release the extractor handle slowly being careful not to lose cells.
- During removal of supernatant be careful not to lose cells.

Worksheet equation:
$A_8 = A_7 - A_1$

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Adjust the weight of the Cell Preparation Bag by adding buffer. Calculate the weight of the Cell Preparation Bag filled with diluted leukapheresis product using the equation below. Record the weight on the worksheet (A_9)

Worksheet equation:
$A_9 = 95\ g + A_1$

Target weight of of Cell Preparation Bag filled with diluted leukapheresis product (g)	=	95 g	+	Weight of empty Cell Preparation Bag (g)

2. Insert the spike of a transfer set to a port of a buffer bag. Confirm the clamp on the plasma transfer set is closed.

3. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

4. Tare the balance. Place the Cell Preparation Bag on the balance.

5. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and fill the Cell Preparation Bag with buffer until the calculated "Target weight of Cell Preparation Bag filled with diluted leukapheresis product" is reached. To stop the liquid flow, close the clamp on the plasma transfer set. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

Important

To ensure maximum depletion of non-target cells from the final product complete resuspension of cell pellet is of highest importance.

7. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

8. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight on the worksheet (A_{10}).

9. Determine the weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag (A_1) from the weight of the filled Cell Preparation Bag (A_{10}). Record the calculated weight on the worksheet (A_{11}).

Worksheet equation:
$A_{11} = A_{10} - A_1$

Weight of leukapheresis product after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

10. Keep the Plasma Waste Bag until the separation and final analysis of all cells has been completed.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

IV. Magnetic labeling of the cells

One vial (7.5 mL) CliniMACS CD45RA Reagent is ready to use and sufficient for one application as described below. The reagent is not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze. The reagent is to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent are printed on the reagent vial. DO NOT use the reagent after the use-by date.

Incubation with CliniMACS® CD45RA Reagent

1. Record the lot number and use-by date of the CliniMACS® CD45RA Reagent on the worksheet.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from one CliniMACS CD45RA Reagent vial (7.5 mL). A 10 mL syringe is sufficient to remove the contents of one vial. The syringe should be equipped with a 20 gauge needle.

3. Using the injection port on the sampling site coupler, inject the entire volume of reagent into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

5. Place the Cell Preparation Bag flat on the orbital rotator, set to a speed of approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Removal of excess reagent

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 500 mL of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag tubing to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer. To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of the filled Cell Preparation Bag on the worksheet (B_1).

5. Close the locking forceps on the Cell Preparation Bag tubing. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag on the balance. Tare the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

14. Open the locking forceps next to the Cell Preparation Bag tubing. Using the plasma extractor, remove as much excess supernatant as possible from the Cell Preparation Bag (B_2).

15. Close the locking forceps and using the heat sealer, seal off the tubing to disconnect the Wash Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.

16. Keep the Wash Waste Bag until the separation and final analysis of all cells has been completed.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Weigh the Cell Preparation Bag and record the weight (B_3). Determine the weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag (A_1) from the weight of the Cell Preparation Bag after the wash (B_3). Record the weight on the worksheet.

Weight of leuka-pheresis product after the wash (g)	=	Weight of filled Cell Pre-paration Bag (g)	–	Weight of empty Cell Pre-paration Bag (g)	Worksheet equation: $B_4 = B_3 - A_1$

19. Adjust sample loading volume: Calculate the amount of buffer necessary to adjust the weight of the cell suspension to approximately 125 g. For loading the labeled and washed cells on the tubing set, a maximum cell concentration of 0.4×10^9 cells per mL should not be exceeded.

Weight of buffer to be added (g)	=	125 g	–	Weight of leuka-pheresis product afterwash (g)	Worksheet equation: $B_5 = 125\ g - B_4$

20. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

21. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

22. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer (B_5) to the Cell Preparation Bag.

23. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

Note

- For loading the labeled and washed cells on the Tubing Set, a maximum cell concentration of 0.4×10^9 cells per mL should not be exceeded.
- Keep the wash waste until the cell separation run has been completed.

24. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

25. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (should include patient identification) and retain for cell analysis. WBC content, CD45RA enumeration and viability testing are recommended.

26. Weigh the Cell Preparation Bag (B_6) and record the weight on the worksheet. Determine the weight of the leukapheresis product (B_7) by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag after buffer addition. Record the weight on the worksheet.

Worksheet equation:
$B_7 = B_6 - A_1$

Weight of leukapheresis product after buffer addition (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Worksheet for preparation and depletion of CD45RA positive cells

Patient Data

_____

_____

	Sample data	Operator
Volume leukapheresis product: mL	mL	
WBC concentration:	cells/mL	Total WBC:
Percentage of CD45RA positive cells:	%	

Transfer of leukapheresis product into the Cell Preparation Bag

Starting sample preparation: Date _____ Time _____

Remove leukapheresis product sample (0.5 mL) for cell count and flow cytometry (label LEUKAPHERESIS PRODUCT). Record analysis data in the table on page 5. Weigh the empty Cell Preparation Bag and transfer the leukapheresis product into the bag. Record the weights of the empty and the filled bag and determine the weight of the transferred leukapheresis product. Do not use apheresis volumes higher than 200 mL.

Weight of empty Cell Preparation Bag
(with locking forceps and sampling site coupler) _____ g (A_1)

Weight of Cell Preparation Bag
filled with leukapheresis product _____ g (A_2)

Weight of leukapheresis product
(difference between filled and empty
Cell Preparation Bag): $A_3 = A_2 - A_1$ _____ g (A_3)

Dilution of leukapheresis product

Dilute the leukapheresis product 1:3 with CliniMACS PBS/EDTA Buffer supplemented with HSA to a final concentration of 0.5% (w/v) before magnetic labeling. Calculate the amount of buffer to be added using the equation below .

Weight of buffer to be added
$A_4 = A_3 \times 2$ _____ g (A_4)

Weight of filled Cell Preparation Bag
after dilution _____ g (A_5)

Weight of diluted leukapheresis product
$A_6 = A_5 - A_1$ _____ g (A_6)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Centrifugation

Centrifugation conditions

Time:
g Force:
Temperature:

Volume adjustment

One vial CliniMACS CD45RA Reagent (7.5 mL) is capable of labeling up to 20×10^9 CD45RA positive cells out of a total leukocyte number of up to 50×10^9 WBCs. Optimal labeling requires a sample volume of 95 mL (+/– 5 mL) and one vial CliniMACS CD45RA Reagent.

Remove as much supernatant as possible. Take care not to resuspend the cell pellet.

Weight of Cell Preparation Bag
after removal of supernatant _____ g (A_7)

Weight of leukapheresis product:
after removal of supernatant: $A_8 = A_7 - A_1$ _____ g (A_8)

Calculate the target weight of the filled Cell Preparation Bag to be reached by adding buffer to give a final sample volume of 95 g using the equation below.

Target weight of filled Cell Preparation Bag
after removal of supernatant: $A_9 = 95\ g + A_1$ _____ g (A_9)

Weight of <u>filled</u> Cell Preparation Bag
after volume adjustment _____ g (A_{10})

Weight of leukapheresis product after
volume adjustment: $A_{11} = A_{10} - A_1$ _____ g (A_{11})

Magnetic labeling of CD45RA positive cells

Lot number of the CliniMACS CD45RA Reagent: _____

Use-by date of the CliniMACS CD45RA Reagent: _____

Resuspend the cell pellet and add the entire content of one vial CliniMACS CD45RA Reagent. Mix carefully and incubate the Cell Preparation Bag for 30 min at room temperature on an orbital shaker at a maximum of 25 rpm.

Incubation

Incubation start time _____

Incubation stop time _____

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Removal of excess reagent

Add CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) to a final volume of 600 mL for cell washing. Weigh the filled Cell Preparation Bag prior to centrifugation and record the weight below.

Weight of filled Cell Preparation Bag _____ g (B_1)

Centrifugation conditions

Time:
g Force:
Temperature:

Remove as much supernatant as possible. Take care not to resuspend the cell pellet.

Amount of removed supernant _____ g (B_2)

Weight of filled Cell Preparation Bag
after washing procedure _____ g (B_3)

Weight of leukapheresis product
after washing procedure
$B_4 = B_3 - A_1$ _____ g (B_4)

Final sample preparation

Adjust the volume of the leukapheresis product to approximately 125 mL for loading on the CliniMACS Depletion Tubing Set (cell concentration should not exceed 0.4×10^9 WBCs/mL). Calculate the amount of buffer to be added using the equation below. Remove leukapheresis product sample (0.5 mL) for cell count and flow cytometry analysis (label ORIGINAL). Record all analysis data in table 1 on page 5.

Amount of buffer to be added
to reach approx. 125 mL
$B_5 = 125\ g - B_4$ _____ g (B_5)

Weight of filled Cell Preparation Bag
after buffer addition _____ g (B_6)

Weight of leukapheresis product
after buffer addition
(sample loading volume)
$B_7 = B_6 - A_1$ _____ g (B_7)
(should be approximately 125 g)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

CliniMACS Separation (Run)

Start: Date _____ Time _____

Separation program used **DEPLETION 3.1**

Lot number of tubing set _____

Expiration date of tubing set _____

Input of sample parameters:

WBC concentration _____ × 10^6/mL

Percentage of labeled cells _____ %

Sample loading volume _____ mL

- After selection of the CliniMACS Depletion Tubing Set (DTS) and program DEPLETION 3.1 enter WBC concentration, frequency of labeled cells and sample loading volume.
  The DTS is provided with an attached Cell Collection Bag. The weight of the empty Cell Collection Bag is **32 g**.
  Check luer lock connections to bags. Luer lock must be closed tightly
- Connect the DTS to the ClinMACS plus Instrument and attach the buffer bag and Cell Preparation Bag following the instructions on the screen. Prime the system and perform the integrity tests. For the integrity test of the upper part of the tubing set, clamp the tubing underneath the check valve of the Non-Target Cell Bag. Remove clamp after completion of the integrity test.
- Before starting the separation confirm the bags are levelled correctly on the hangers:
  - Highest position: buffer bag,
  - Middle position: Reapplication and Non-Target Cell Bag,
  - Lowest position: Cell Preparation Bag.
- Press 'RUN' to start the separation.
- Record the instrument process code after the run.

Process code _____

Calculation of the amount of target cell fraction (CD45RA depleted fraction)

Weight of empty Cell Collection Bag _____ g (C_1)

Weight of Cell Collection Bag after the run has been finished _____ g (C_2)

Weight of target cell fraction ($C_3 = C_2 - C_1$) _____ g (C_3)

Remove sample for cell count and flow cytometric analysis (label **Target Cell Fraction**).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

CliniMACS® Worksheet

Calculation of the amount of non-target cell fraction

Weight of empty Negative Fraction Bag _____ g (C_4)

Weight of Negative Fraction Bag after the run has been finished _____ g (C_5)

Weight of non-target cell fraction: $C_6 = C_5 - C_4$ _____ g (C6)

Remove sample for cell count and flow cytometric analysis (label **Non-Target Cell Fraction**).

Optional: Calculation of the amount of wash fraction (Buffer Waste Bag)

Weight of empty Buffer Waste Bag _____ g (C_7)

Weight of Buffer Waste Bag after the run has been finished _____ g (C_8)

Weight of buffer waste: $C_9 = C_8 - C_7$ _____ g (C_9)

Remove sample for cell count and flow cytometric analysis (label **Buffer Waste**).

Data analysis

For details regarding the analysis of CD45RA positive T cells after separation, please refer to the following Special Protocol: "*Proposal for CD45RA positive cell determination by flow cytometry after depletion of CD45RA positive cells*."

The table below summarizes analysis data required. Further analysis of additional parameters may be included based on the intended use of the target cells.

Fraction	Volume (mL)	Total cells (10^9)	$CD3^+/CD45RA^+$ (%)	Total no. $CD3^+/CD45RA^+$	$CD3^+/CD45RA^-$ (%)	Total no. $CD3^+/CD45RA^-$	Viability (%)
Leukapheresis Product							
Original fraction (after sample preparation)							
Target cells, CD45RA depleted (Cell Collection Bag)							
Non-target cells (Negative Fraction Bag)							
Optional: Wash waste (Buffer Waste Bag)							

Yield of target cells (CD45RA negative cells) _____

Log Depletion _____

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® CD271 Reagent (Bone marrow)

I. General information, warnings and precautions

General information

The CliniMACS® Plus CD271 System including the CliniMACS plus Instrument, the CliniMACS CD271 Reagent, the CliniMACS Tubing Set TS, and CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human CD271 positive cells from bone marrow aspirate.

The CD271 antigen, also known as low-affinity nerve growth factor receptor, is expressed on mesenchymal stromal cells (MSC) and can be used for the direct enrichment of these cells from bone marrow aspirate.

The CliniMACS Plus CD271 System uses selective CD271 monoclonal antibodies conjugated to super-paramagnetic particles. The CD271 positive cells are specifically labeled by incubation with the CliniMACS CD271 Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the separation in an automated continuous flow separation process.

For the enrichment of CD271 positive cells, the separation program ENRICHMENT 3.2 must be used.

The CliniMACS Plus CD271 System passes antibody-labeled suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled CD271 positive cells (target cells) while the non-labeled cells flow through the column. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged, and the magnetic field is removed. The target cells are collected into the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, cellular starting product (bone marrow aspirate), CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® CD271 Reagent

Intended purpose

- The CliniMACS® CD271 Reagent is intended for the *in vitro* enrichment of human CD271 positive cells from heterogeneous hematologic cell populations in combination with the CliniMACS System only.

- The CliniMACS Plus CD271 System is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS CD271 Reagent, the CliniMACS Tubing Set TS, and CliniMACS PBS/EDTA Buffer.

Composition

Each vial of CliniMACS CD271 Reagent contains 7.5 mL of a colloidal solution of iron-dextran beads conjugated to murine monoclonal CD271 antibody in PBS/EDTA buff er stabilized with Poloxamer 188 (0.03% w/v).

Side-effects

- When the enriched CD271 positive cells are injected or infused into patients, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndromes. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease could be more susceptible to iron-dextran reactions.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Precautions

- Before use in human applications, the suitability of the target cells must be demonstrated regarding indication, quality and quantity.
- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively within the responsibility of the user.
- The enrichment of CD271 positive cells using the CliniMACS CD271 Reagent must be performed by trained operators only.
- Operators using the CliniMACS CD271 Reagent should have experience in the separation of cells from bone marrow or peripheral blood.
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- For *in vitro* use only. Do not inject or infuse the reagent directly into the patient. Not for parenteral application.
- The CliniMACS CD271 Reagent is not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The CliniMACS CD271 Reagent is for single use only (see fig. 4.1-1).
- Do not use the reagent after the use-by date printed on the vial label (see fig. 4.1-2).
- Do not use the reagent if package is damaged (see fig. 4.1-3). Use undamaged and sealed vials only.
- Store at +2 °C to +8 °C (see fig. 4.1-4). Do not freeze.

Further information

- The CliniMACS CD271 Reagent is tested for endotoxins.
- The reagent is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

Important

This protocol requires the use of components which are not part of the CliniMACS Plus CD271 System (e.g., HSA). Therefore, all materials used must be pharmaceutical grad or FDA-licensed for human use.

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

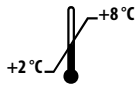


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Fig. 4.1-5: Sterilized using aseptic processing techniques.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

II. First actions required

CliniMACS® Materials required

Note

The application capacity for the enrichment of CD271 positive cells using the CliniMACS Plus CD271 System is 100 mL of bone marrow aspirate and a maximum of 1×10^7 CD271 positive cells.

- **CliniMACS® CD271 Reagent, REF 701-12.**
  One vial is required for the application using the CliniMACS Plus CD271 System. If the labeling volume must be adjusted to 190 mL, a second CliniMACS CD271 Reagent must be used.

- **CliniMACS Tubing Set TS, REF 161-01**.
  One CliniMACS Tubing Set TS must be used for the enrichment of CD271 positive cells.

- **CliniMACS PBS/EDTA Buffer, REF 700-25.**
  CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. For the preparation procedure, one liter of buffer is required. For the separation, one liter of buffer is required. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Additional materials required

- **Cell Preparation Bag:**
  One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and one plasma transfer set, for use during the cell preparation procedure.

- **Plasma Waste Bag and Wash Waste Bag:**
  Two 600 mL transfer bags, suitable for centrifugation.

- **Bone Marrow Aspirate Bag:**
  One 600 mL transfer bag in combination with a Luer/Spike Interconnector.

- **Cell Collection Bag:**
  One 150 mL transfer bags in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set.

- Pre-system filter.

- One 200 µm in-line blood filter.

- Locking forceps or slide clamps.

- Appropriate syringes (1 mL, 10 mL, 20 mL) and hypodermic 20 gauge needles.

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).

- Sample tubes.

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3: "Equipment required".

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 "Additional materials required".

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- Note that **HSA is not a component of the CliniMACS Plus System**. Use only FDA licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.
- Store the buffer for cell preparation at +19 °C to +25 °C. Lower or higher ambient temperature will result in less purity and yield of the target cells.
- Since length of the coupler can vary during the preparation procedure be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement each required liter of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5 % (w/v), i.e., add 5 g HSA per liter buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

- Label one 150 mL transfer bag as: **Cell Collection**. (This should include patient identification, date and time of run, and operator identification.) Insert a Luer/Spike Interconnector into the port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL transfer bag as: **Bone Marrow Aspirate**. (This should include patient identification, date and time of run, and operator identification.) Insert a Luer/Spike Interconnector into the outside port of the Bone Marrow Aspirate Bag.

- Label one 600 mL transfer bag as: **Cell Preparation**. (This should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL transfer bag as: **Plasma Waste**. (This should include patient identification, date, time of run, and operator identification.)

- Label one 600 mL transfer bag as: **Wash Waste**. (This should include patient identification, date and time of run, operator identification.)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

III. Bone marrow aspirate

The following sections describe the recommended procedure for the preparation of the bone marrow aspirate using the Terumo Sterile Connection Device (TSCD).

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Analysis of bone marrow aspirate

Before starting the preparation at least the following parameters should be determined:

- Volume and cell concentration to determine the total number of leukocytes,
- CD271 enumeration,
- Viability.

Other tests might be required depending on the intended use of the cells or individual laboratory practice. Record all data.

Important

- All bag handling should be performed in a sterile environment (e.g. laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

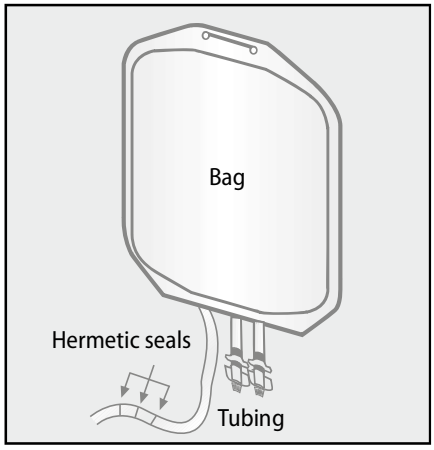


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

Filtration of bone marrow aspirate and transfer into Cell Preparation Bag

1. Record the date and the start time before beginning with the procedure.

2. Combine all bone marrow aspirates by transferring each aspirate into the Bone Marrow Aspirate Bag through the luer lock interconnector or by using the TSCD.

3. Holding the Bone Marrow Aspirate Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

4. Using the TSCD, connect the filled Bone Marrow Aspirate Bag to one site of the in-line blood filter, and the Cell Preparation Bag to the other site of the filter. Secure the tubing with clamps to close the fluid pathway.

5. Open the fluid pathway and hold the Bone Marrow Aspirate Bag up to let the bone marrow aspirate run through the in-line blood filter into the Cell Preparation Bag. Use a tubing stripper to clear the tubing from any remaining bone marrow aspirate. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing and separate bags, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see fig. 4.1-6). Retain the Bone Marrow Aspirate Bag until cell separation and final analysis have been completed.

7. Determine the volume of the filtered bone marrow aspirate by estimating 1 mL of cellular product as equivalent to 1 g (1 g = 1 mL).

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the weight of the filtered bone marrow aspirate by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with bone marrow aspirate. Record the weight.

Weight of filtered bone marrow aspirate (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

10. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the bone marrow aspirate. Transfer the sample into a sample tube. Label the tube as BONE MARROW ASPIRATE (should include patient identification) and retain for cell analysis. WBC content, CD271enumeration and viability testing are recommended.

Dilution of bone marrow aspirate

The bone marrow aspirate must be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Calculate the weight of buffer to be added using the following equation and record it.

$$\text{Weight of buffer to be added (g)} = \text{Weight of bone marrow aspirate (g)} \times 4$$

1. Take a plasma transfer set and ensure the clamp is closed. Insert the spike of the plasma transfer set into a port of the buffer bag.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

4. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer to the Cell Preparation Bag.

5. When the appropriate weight of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the weight of the weight of buffer added.

6. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Weigh the Cell Preparation Bag and record the weight.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

9. Determine the weight of diluted bone marrow aspirate by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of diluted bone marrow aspirate (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 300×g for 15 min (without brake) at controlled room temperature (+19 °C to +25 °C). Record centrifugation conditions.

5. Remove the bag from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Volume adjustment

1. For magnetic labeling of CD271 positive cells, the optimal weight of the cell sample must be adjusted to 95 g (± 5 g).

   Calculate the weight of supernatant to be removed to adjust the sample to 95 g using the equation below:

Weight of supernatant to be removed (g)	=	Weight of diluted bone marrow aspirate (g)	–	95 g

   Record the weight of supernatant to be removed.

2. Place the empty Plasma Waste Bag on the balance and tare the balance.

3. Open locking forceps next to the Cell Preparation Bag. Use the balance display to monitor the weight of the Plasma Waste Bag while removing the supernatant. Carefully press out excess diluted plasma using the plasma extractor. Continue until the calculated weight of supernatant has been transferred into the Plasma Waste Bag so that 95 g remain in the Cell Preparation Bag. If necessary, stop the supernatant removal before cells get transferred into the Plasma Waste Bag.

4. Close the locking forceps next to the Cell Preparation Bag when the calculated weight of supernatent in the Plasma Waste Bag has been reached. Record the weight of the supernatant removed.

5. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag.

6. Weigh the Cell Preparation Bag and record the weight.

7. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

8. Determine the weight of the bone marrow aspirate after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of bone marrow aspirate after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

9. **If the weight of the bone marrow aspirate exceeds 95 g (± 5 g), the sample must be adjusted to 190 g (± 5 g). If not, please proceed to number 19 of the protocol.**

Important

- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps on the Cell Preparation Bag is in the open position before beginning the transfer. Release the extractor handle slowly.
- During removal of supernatant be careful not to lose cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

10. Calculate the weight of buffer to be added to adjust the bone marrow aspirate to 190 g (± 5 g) using the following equation and record it.

Weight of buffer to be added (g)	=	190 g	–	Weight of bone marrow aspirate (g)

11. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

12. Place the Cell Preparation Bag on the balance and tare the balance.

13. Open locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer to the Cell Preparation Bag.

14. When the appropriate weight of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the weight of buffer added.

15. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

16. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

17. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

18. Determine the weight of the bone marrow aspirate after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag Record the calculated weight.

Weight of bone marrow aspirate after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

19. Keep the Plasma Waste Bag until the separation and final analysis of all cells has been completed.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

IV. Magnetic labeling of the cells

One vial (7.5 mL) CliniMACS CD271 Reagent is ready to use and sufficient for one application as described below. The reagent is not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze. The reagent must be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent are printed on the reagent vial. DO NOT use the reagent after the use-by date.

Incubation with CliniMACS® CD271 Reagent

1. Record the lot number and use-by date of the CliniMACS® CD271 Reagent.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from one CliniMACS CD271 Reagent vial (7.5 mL each). A 10 mL syringe is sufficient to remove the contents of one reagent vial or a 20 mL syringe is sufficient to remove the contents of two reagent vials, respectively. The syringe should be equipped with a 20 gauge needle.

3. **If the labeling volume of the bone marrow aspirate was adjusted to 190 g (±5 g), a second vial of CliniMACS CD271 Reagent must be used.**

4. Using the injection port on the sampling site coupler, inject the entire volume of reagent into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation.

5. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

6. Place the Cell Preparation Bag flat on the orbital rotator, set to a speed of approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 min at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Removal of excess reagent

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 500 mL of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer (i.e. add 400 g to 500 g of buffer). To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of buffer transferred into the Cell Preparation Bag.

5. Close the locking forceps next to the Cell Preparation Bag. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (bone marrow aspirate and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C). Record the centrifugation conditions.

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag on the balance. Tare the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

14. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much supernatant as possible from the Cell Preparation Bag. Record the weight of removed supernatant.

15. Close the locking forceps next to the Cell Preparation Bag. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Wash Waste Bag.

16. Keep the Wash Waste Bag until the cell separation and final analysis of all cells has been accomplished.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the bone marrow aspirate after the wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag. Record the weight.

Weight of bone marrow aspirate after the wash (g)	=	Weight Cell Preparation Bag after the wash (g)	–	Weight of empty Cell Preparation Bag (g)

19. If the determined weight ranges between 100 g and 275 g, proceed to number 26. If the weight is less than 100 g, adjust sample loading volume: Calculate the weight of buffer necessary to adjust the weight of the cell suspension to approximately 100 g. The weight must not exceed 275 g.

Weight of buffer to be to be added (g)	=	100 g	–	Weight of bone marrow aspirate after the wash (g)

20. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

21. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

22. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer to the Cell Preparation Bag.

Important

- For the normal scale preparation (one vial of CliniMACS CD271 Reagent), the volume of the removed supernatant should be at least 500 mL.
- If the supernatant removed is less than 500 mL, a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

23. When the appropriate weight of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

24. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

25. Resuspend the cells in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

26. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (should include patient identification) and retain for cell analysis. WBC content, CD271 enumeration and viability testing are recommended.

27. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the bone marrow aspirate after the addition of buffer (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of bone marrow aspirate after buffer addition (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 1:

CliniMACS® CD62L Reagent

I. General information, warnings and precautions

General information

The CliniMACS® Plus CD62L System including the CliniMACS ^{plus} Instrument, the CliniMACS CD62L Reagent, the CliniMACS Tubing Set LS, and CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human CD62L positive cells from heterogeneous hematologic cell populations.

The human CD62L antigen is a 74 kDa glycoprotein and a member of the selectin family of cell surface molecules. CD62L is also known as L-selectin, LECAM-1, or LAM-1 and binds a series of glycoproteins including CD34, GlyCAM-1, and MAdCAM-1. CD62L is important for homing of naive lymphocytes via endothelial venules to peripheral lymph nodes and Peyer's patches. The CD62L antigen also contributes to the recruitment of leukocytes from the blood to areas of inflammation. Most hematopoietic cells express CD62L, including most peripheral blood B cells, T cells, monocytes, granulocytes, some myeloid cells from bone marrow, and thymocytes. CD62L is continuously endoproteolytically cleaved from the cell surface neutrophils and lymphocytes. Proteolysis is accelerated, for example, after antigenic activation of T cells.

The CliniMACS Plus CD62L System uses selective CD62L monoclonal antibodies conjugated to super-paramagnetic particles. The CD62L positive cells are specifically labeled by incubation with the CliniMACS CD62L Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the enrichment in an automated continuous flow separation process.

The CliniMACS Plus CD62L System passes the antibody-labeled suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled CD62L positive target cells while unwanted cells (non-target cells) flow through the column and are collected in the Negative Fraction Bag. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged, and the magnetic field is removed. The target cells are collected in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, cellular starting product, CliniMACS Tubing Sets, and CliniMACS PBS/EDTA Buffer, please refer to the applicable section in chapter 3.

CliniMACS® CD62L Reagent

Intended purpose

- The CliniMACS® CD62L Reagent is intended for the *in vitro* enrichment of human CD62L positive cells from heterogeneous hematologic cell populations in combination with the CliniMACS System only.

- The CliniMACS Plus CD62L System is comprised of the CliniMACS ^{plus} Instrument, the CliniMACS CD62L Reagent, the CliniMACS Tubing Set LS, and CliniMACS PBS/EDTA Buffer.

Composition

Each vial of CliniMACS CD62L Reagent contains 7.5 mL of a colloidal solution of iron-dextran beads conjugated to murine monoclonal CD62L antibody in PBS/EDTA buff er stabilized with Poloxamer 188 (0.03% w/v).

Side-effects

- When the enriched CD62L positive cells are infused or injected into patients, they may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available.

- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndromes. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease could be more susceptible to iron-dextran reactions.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Precautions

- Before use in human applications, the suitability of target cells must be demonstrated regarding indication, quality and quantity.
- Further processing and use for clinical applications must be in accordance with FDA investigational use regulations.
- Any clinical application of the separated cells is exclusively within the responsibility of the user.
- The enrichment of CD62L positive cells using the CliniMACS CD62L Reagent must be performed by trained operators only.
- Operators using the CliniMACS CD62L Reagent should have experience in the separation of cells from bone marrow or peripheral blood.
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed.

Warnings

- The CliniMACS CD62L Reagent is for *in vitro* use only. Do not inject or infuse the reagent directly into the patient. Not for parenteral application.
- The reagent is not recommended for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Patients may develop human anti-mouse antibodies (HAMA).
- The reagent is for single use only (see fig. 4.1-1).
- Do not use the reagent after the use-by date printed on the vial label (see fig. 4.1-2).
- Do not use the reagent if package is damaged (see fig. 4.1-3). Use undamaged and sealed vials only.
- Store at +2 °C to +8 °C (see fig. 4.1-4). Do not freeze.

Further information

- The CliniMACS CD62L Reagent is tested for endotoxins.
- The reagent is sterile (see fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically.

Important

This protocol requires the use of components which are not part of the CliniMACS Plus CD62L System (e.g., HSA). Therefore all materials used must be pharmaceutical grade of FDA-licensed for human use.

Fig. 4.1-1: Do not re-use.

Fig. 4.1-2: Use-by date

Fig. 4.1-3: Do not use if package is damaged.

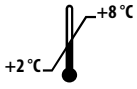


Fig. 4.1-4: Temperature limit +2 °C to +8 °C

Fig. 4.1-5: Sterilized using aseptic processing techniques.

II. First actions required

Note

The application capacity for the enrichment of CD62L positive cells using the CliniMACS Plus CD62L System is 4×10^9 CD62L positive cells out of a total cell number not exceeding 10×10^9 nuclated cells.

CliniMACS® Materials required

- **CliniMACS® CD62L Reagent, REF 701-19.**
  One vial is required for the application using the CliniMACS Plus CD62L System.

- **CliniMACS Tubing Set LS, REF 162-01**.
  One CliniMACS Tubing Set LS must be used for the enrichment of CD62L positive cells.

- **CliniMACS PBS/EDTA Buffer, REF 700-25.**
  CliniMACS PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS Plus Separation. For the preparation procedure, one liter of buffer is required. For the separation, up to three liters of buffer are required (see table 4.1-1). Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v). For the whole preparation procedure use pre-cooled buffer at +2 °C to +8 °C.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Additional materials required

- **Cell Preparation Bag:**
  One 600 mL transfer tag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure.

- **Plasma Waste Bag and Wash Waste Bag:**
  Two 600 mL transfer bags, suitable for centrifugation.

- **Cell Collection Bag:**
  One 600 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set.

- **If the replacement of the Negative Fraction Bag and the Buffer Waste Bag is necessary (see table 4.1-1):**
  Two 1000 mL transfer bags in combination with two Luer/Spike Interconnectors to connect the bags to the tubing set.

- **If more than one liter of buffer is required for the separation (see table 4.1-1):**
  One to two plasma transfer sets to connect the buffer bags.

- Pre-system filter.

- Locking forceps or slide clamps.

- Appropriate syringes (1 mL, 10 mL) and hypodermic 20 gauge needles.

- Human serum albumin (HSA) to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v).

- Sample tubes.

Equipment required

Please find information regarding equipment required for a CliniMACS Plus Separation in chapter 3 "Equipment required".

Note

For detailed information regarding additional materials suggested for a CliniMACS Plus Separation, please refer to chapter 3 "Additional materials required".

Important

After input of sample parameters (STEP 2), the CliniMACS Software calculates the volumes that will be collected in the Priming Waste Bag, Cell Collection Bag, Negative Fraction Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volume of 500 mL, replacement of bags according to the following overview of materials required is necessary.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Overview of materials required

Cell preparation		
Total cells (WBCs)	10×10^9	
Labeled cells	$\leq2\times10^9$	$2–4\times10^9$
Number of CliniMACS CD62L Reagent vials	1	1
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	1 liter
Plasma transfer set	1	1
CliniMACS Plus Separation		
Negative Fraction Bag	-	Replace original bags of the tubing set by 1000 mL transfer bags
Buffer Waste Bag	-	
Priming Waste Bag	-	-
Cell Collection Bag	600 mL	600 mL
Luer/Spike Interconnector	1	3
CliniMACS PBS/EDTA Buffer (suppl. with HSA to a final concentration of 0.5% (w/v))	1 liter	2–3 liters
Plasma transfer set	-	1-2

Table 4.1-1: Materials required for cell preparation and separation of CD62L positive cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Preparation of the CliniMACS PBS/EDTA Buffer

Supplement each required liter of CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v), i. e. add 5 g HSA per liter buffer.

After the CliniMACS Plus Separation, the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans prior to target cell infusion. The user of the CliniMACS Plus System is responsible for the selection of an appropriate medium for infusion.

Labeling and preparation of bags

- Label one 600 mL transfer bag as: **Cell Collection**. (This should include patient identification, date and time of run, and operator identification.) Insert a Luer/Spike Interconnector into the port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL transfer bag as: **Cell Preparation**. (This should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

- Label one 600 mL transfer bag as: **Plasma Waste**. (This should include patient identification, date, time of run, and operator identification.)

- Label one 600 mL transfer bag as: **Wash Waste**. (This should include patient identification, date and time of run, operator identification.)

Important

- Note that **HSA is not a component of the CliniMACS Plus System**. Use only FDA licensed HSA. Carefully read the package insert of the HSA used. All risks arising from these materials must be evaluated by the user.

- For the whole preparation procedure use pre-cooled buffer at +2 °C to +8 °C. Pre-cool the buffer before starting the cell preparation procedure. Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the coupler can vary during the preparation procedure be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are lying on the balance and the rest of the tubing is lying on the table next to the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- All bag handling should be performed in a sterile environment (e.g., laminar flow hood) using aseptic techniques. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

III. Leukapheresis product

The following sections describe the recommended procedure for the preparation of the leukapheresis product using the Terumo Sterile Connection Device (TSCD).

- The operator must be familiar with the operation and use of the TSCD.

- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Analysis of leukapheresis product

Before starting the preparation of the leukapheresis product the following parameters should be determined.

- Total number of leukocytes,
- Percentage of CD62L positive cells,
- Total number of CD62L positive cells,
- Viability.

Recheck the parameters after the labeling procedure. This is necessary because the following three sample parameters must be entered in the software during the setup of the CliniMACS ^{plus} Instrument prior to loading the tubing set onto the instrument:

- Concentration of leukocytes per mL,
- Percentage of CD62L positive cells,
- Final volume of the cell sample (sample loading volume).

Other tests might be required depending on the intended use of the cells (e.g. phenotype of T cells) or according to individual laboratory practice. Record all data.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Transfer of leukapheresis product into Cell Preparation Bag

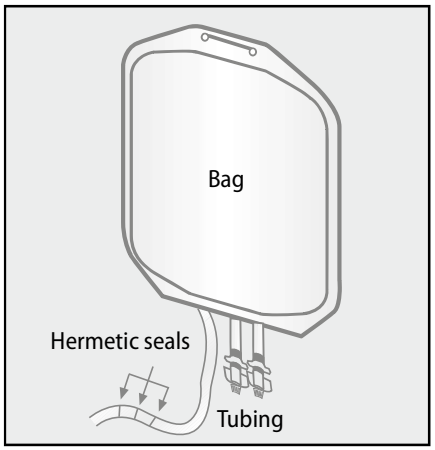


Fig. 4.1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

1. Record the date and the start time before beginning to prepare the leukapheresis product.

2. Determine the volume of the original leukapheresis product by estimating 1 mL of leukapheresis product as equivalent to 1 g (1 g = 1 mL).

3. Holding the leukapheresis product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.

4. Using the TSCD, connect the Cell Preparation Bag to the original leukapheresis product bag.

5. Open the clamp to transfer the leukapheresis product material into the Cell Preparation Bag. Use a tubing stripper to clear the tubing from any remaining luekapheresis product. Close the clamp next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing between both clamps, leaving at least 15 cm of tubing on the Cell Preparation for further connections (see fig. 4.1-6). Retain the original leukapheresis bag until cell separation and final cell analysis have been completed.

7. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the leukapheresis product. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS PRODUCT (this should include patient identification) and retain for cell analysis. Minimally, analysis of the WBC content, CD62L enumeration and viability testing are recommended.

8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight of the filled Cell Preparation Bag.

9. Determine the weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with leukapheresis product. Record the weight.

Weight of leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

- **For the whole preparation procedure use pre-cooled buffer at +2 °C to +8 °C.** Pre-cool the buffer before starting the cell preparation procedure.

Dilution of leukapheresis product

The leukapheresis product must be diluted with pre-cooled CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Dilute the leukapheresis product with buffer to a total volume of 600 mL. Calculate the amount of buffer to be added using the following equation and record it.

Weight of buffer to be added (g)	=	600 g	–	Weight of leukapheresis product (g)

1. Take a plasma transfer set and ensure the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

4. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the weight of the filled Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.

8. Determine the total weight of diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of diluted leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Centrifugation

1. Using the TSCD,connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Volume adjustment

1. For magnetic labeling of CD62L positive cells, the optimal weight of the cell sample is 95 g (±5 g).

   Remove supernatant completely, taking care not to resuspend the cell pellet during removal of supernatant. Slide the clamp on the plasma transfer bag to the closed position, when the supernatant has been removed. Using the TSCD, seal off the tubing to disconnect the Plasma Waste Bag. Resuspend the pellet carefully.

   Adjust the weight of the Cell Preparation Bag by adding buffer. Calculate the weight of the Cell Preparation Bag filled with diluted leukapheresis product using the equation below:

Target weight of of Cell Preparation Bag filled with diluted leukapheresis product (g)	=	95 g	+	Weight of empty Cell Preparation Bag (g)

2. Insert the spike of a transfer set to a port of a buffer bag containing 1 liter of buffer. Confirm the clamp on the plasma transfer set is closed.

3. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

Important

- **For the whole preparation procedure use pre-cooled buffer at +2 °C to +8 °C.** Pre-cool the buffer before starting the cell preparation procedure.
- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is in the open position before beginning the transfer. Release the extractor handle slowly.
- During removal of supernatant be careful not to lose cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

4. Tare the balance. Place the Cell Preparation Bag on the balance.

5. Open the locking forceps next to Cell Preparation Bag. Then open the clamp on the plasma transfer set to fill the Cell Preparation Bag with buffer until the calculated "Target weight of Cell Preparation Bag filled with diluted leukapheresis product" is reached. To stop the liquid flow, close the clamp on the plasma transfer set. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

8. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

9. Determine the weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

10. Keep the Plasma Waste Bag until the separation and final analysis of cells has been completed.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

IV. Magnetic labeling of cells

One vial (7.5 mL) CliniMACS CD62L Reagent is ready to use and sufficient for one preparation as described below. The reagent is not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze. The reagent is to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent are printed on the vial. DO NOT use the reagent after the use-by date.

Incubation with the CliniMACS® CD62L Reagent

1. Record the lot number and use-by date of the CliniMACS® CD62L Reagent.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from the CliniMACS CD62L Reagent vial (7.5 mL). A 10 mL syringe is sufficient to remove the contents of one reagent vial. The syringe should be equipped with a 20 gauge needle.

3. Using the injection port on the sampling site coupler, inject the entire volume of reagent into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 15 minute incubation.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

5. Place the Cell Preparation Bag flat on the rotator, set to a speed of approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 15 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Important

For the whole preparation procedure use pre-cooled buffer at +2 °C to +8 °C. Pre-cool the buffer before starting the cell preparation procedure.

Removal of excess reagent

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least one liter of buffer. Confirm the clamp on the plasma transfer set is closed.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the bag hanger.

3. Place the Cell Preparation Bag on the balance. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer (i.e., add 450 g to 550 g of buffer). To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of the filled Cell Preparation Bag. Close the locking forceps next to the Cell Preparation Bag.

5. Using the heat sealer, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.

7. Using the TSCD, connect the empty Wash Waste Bag to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C).

11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.

12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag on the balance. Tare the balance.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

14. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much excess supernatant as possible from the Cell Preparation Bag. Record the amount of removed supernatant. Close the locking forceps next to the Cell Preparation Bag.

15. Using the heat sealer, seal off the tubing to disconnect the Wash Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.

16. Keep the Wash Waste Bag until the separation and final analysis of all cells has been accomplished.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product after the wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Weight of leukapheresis product after the wash (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

19. Adjust sample loading volume: Calculate the amount of buffer necessary to adjust the weight of the cell suspension to approximately 100 g.

Amount of buffer to be to be added (g)	=	100 g	–	Weight of leukapheresis product after the wash (g)

20. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

21. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.

22. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.

Important

- If the supernatant removed is less than 500 mL a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.
- For the preparation the volume of supernatant removed should be at least 500 mL.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

23. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next of the Cell Preparation Bag.

24. Using the heat sealer, seal off the tubing between both clamp. Disconnect the buffer bag.

25. Resuspend the cell pellet in the Cell Preparation Bag. Avoid too intensive mixing of the cells. Ensure that all cells are resuspended.

26. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (This should include patient identification.) and retain for cell analysis. WBC content, CD62L enumeration and viability testing are recommended.

27. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the leukapheresis product (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Note the weight.

Weight of leuka-pheresis product after buffer addition (g)	=	Weight of filled Cell Pre-paration Bag (g)	–	Weight of empty Cell Pre-paration Bag (g)

Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

STEP 2:

CD34 SELECTION 1/2

Switch-on of the CliniMACS® plus Instrument

Switch on the CliniMACS® plus Instrument by using the ON/OFF switch located on the back panel of the instrument. Record the date and start time of the instrument run on the worksheet (C_1).

The window will display screens no. 1.1 and no. 1.2 as shown in chapter 1.3.9.

To proceed to the program menu, press ENT

Note

Screen prompts and diagrams serving as procedure guides will appear in the display window. Perform and check each step according to the manual instructions before proceeding to the next step.

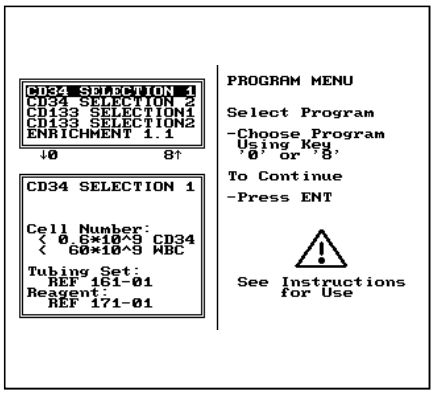
Choice of separation program CD34 SELECTION 1/2

The window will display screen no. 4.2-1 as shown.

Depending on the application, choose CD34 SELECTION 1 or CD34 SELECTION 2 .

To choose the separation program, highlight the name of the program with the black bar. Move the bar up and down by using the keys '0' and '8'.

To proceed with the highlighted program, press ENT



Screen no. 4.2-1

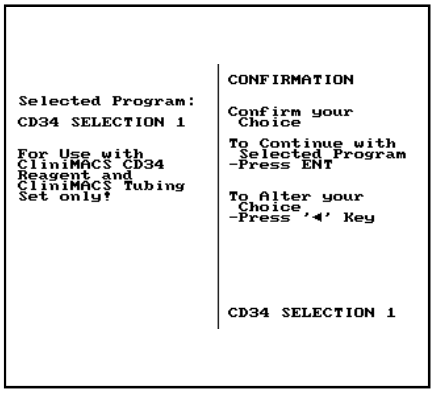
Confirmation of program choice

The window will display screen no. 4.2-2 as shown.

Confirm the correct program has been chosen.

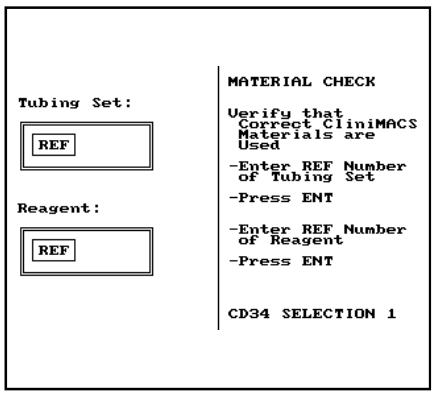
If not, press the "Undo" key (fig. 1.16) to return to the previous step in order to amend the choice.

To confirm and proceed, press ENT



Screen no. 4.2-2

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.2-3

Material check

The window will show screen no. 4.2-3 as shown.

CD34 SELECTION 1 and CD34 SELECTION 2 are optimized for the enrichment of CD34 positive cells.

To confirm the suitable tubing set is available and the proper reagent has been used for cell labeling, enter respective reference number (REF) in the query box. The instrument will check whether the materials can be used in combination with the chosen program.

1. Enter reference number of the tubing set to be used for automated cell separation.

   To confirm and proceed, press ENT

2. Enter reference number of the reagent that has been used for cell labeling.

   To confirm and proceed, press ENT

If the reference number of a tubing set or a reagent not specified for the chosen separation program has been entered, a message appears. Press 'ENT' to confirm and enter the correct reference number. If the reference number entered is still incorrect, the message will appear a second time. After pressing 'ENT' the program will return to the program menu (see screen no. 4.2-1).

If the material check was successful, the program continues automatically with the instructions to install the tubing set.

Proceed to STEP 3.

Note

- CD34 SELECTION 1 must only be used in combination with the CliniMACS Tubing Set TS (REF 161-01), while CD34 SELECTION 2 must only be used in combination with the CliniMACS Tubing Set LS (REF 162-01). Carefully check the tubing set prior to installation.

- To correct a mistake during data input, press the "Undo" key (17, fig. 1.13).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

STEP 2:

CD133 SELECTION 1/2

Switch-on of the CliniMACS® plus Instrument

Switch on the CliniMACS® plus Instrument by using the ON/OFF switch located on the back panel of the instrument. Record the date and and start time of the instrument run on the worksheet (C_1).

The window will display screens no. 1.1 and no. 1.2 as shown in chapter 1.3.9.

To proceed to the program menu, press ENT

Note

Screen prompts and diagrams serving as procedure guides will appear in the display window. Perform and check each step according to the manual instructions before proceeding to the next step.

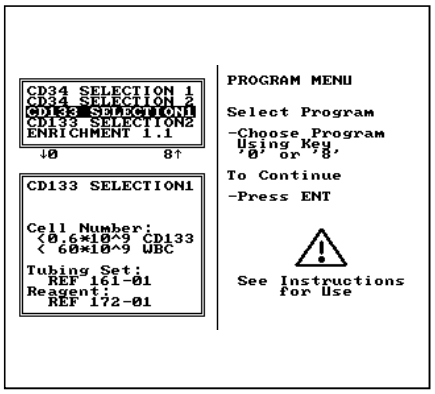
Choice of separation program CD133 SELECTION 1/2

The window will display screen no. 4.2-1 as shown.

Depending on the application, choose CD133 SELECTION 1 or CD133 SELECTION 2 .

To choose the separation program, highlight the name of the program with the black bar. Move the bar up and down by using the keys '0' and '8'.

To proceed with the highlighted program, press ENT



Screen no. 4.2-1

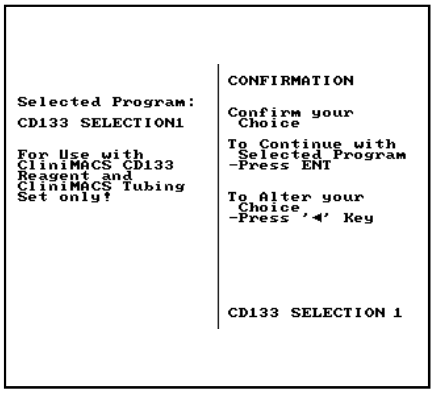
Confirmation of program choice

The window will display screen no. 4.2-2 as shown.

Confirm the correct program has been chosen.

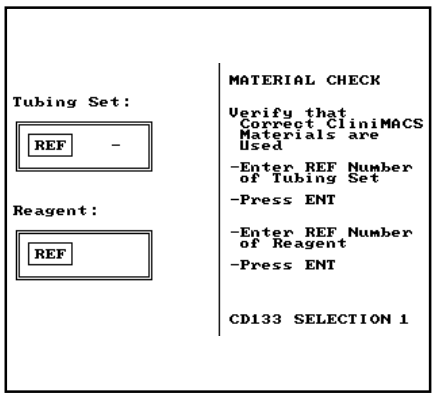
If not, press the "Undo" key (see, fig. 1.16) to return to the previous step in order to amend the choice.

To confirm and proceed, press ENT



Screen no. 4.2-2

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.2-3

Note

- CD133 SELECTION 1 must only be used in combination with the CliniMACS Tubing Set TS (REF 161-01), while CD133 SELECTION 2 must only be used in combination with the CliniMACS Tubing Set LS (REF 162-01). Carefully check the tubing set prior to installation.
- To correct a mistake during data input, press the "Undo" key (see fig. 1.16).

Material check

The window will show screen no. 4.2-3 as shown.

CD133 SELECTION 1 and CD133 SELECTION 2 are optimized for the enrichment of CD133 positive cells.

To confirm the suitable tubing set is available and the proper reagent has been used for cell labeling, enter respective reference number (REF) in the query box. The instrument will check whether the materials can be used in combination with the chosen program.

1. Enter reference number of the tubing set to be used for automated cell separation.

   To confirm and proceed, press ENT

2. Enter reference number of the reagent that has been used for cell labeling.

   To confirm and proceed, press ENT

If the reference number of a tubing set or a reagent not specified for the chosen separation program has been entered, a message appears. Press 'ENT' to confirm and enter the correct reference number. If the reference number entered is still incorrect, the message will appear a second time. After pressing 'ENT' the program will return to the program menu (see screen no. 4.2-1).

If the material check was successful, the program continues automatically with the instructions to install the tubing set.

Proceed to STEP 3.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

STEP 2:

ENRICHMENT 1.1

Switch-on of the CliniMACS® plus Instrument

Switch on the CliniMACS® plus Instrument by using the ON/OFF switch located on the back panel of the Instrument. Record the date and start time of the instrument run.

The window will display screens no. 1.1 and no. 1.2 as shown in chapter 1.3.9.

To proceed to the program menu, press ENT

Choice of separation program ENRICHMENT 1.1

The window will display screen no. 4.2-1 as shown.

Choose ENRICHMENT 1.1.

To choose the separation program, highlight the name of the program with the black bar. Move the bar up and down by using the keys '0' and '8'.

To proceed with the highlighted program, press ENT

Selection of the tubing set

The window will display screen no. 4.2-2 as shown.

Confirm that the correct program has been chosen.

If not, press the "Undo" key (see fig. 1.16) to return to the previous step in order to amend the choice.

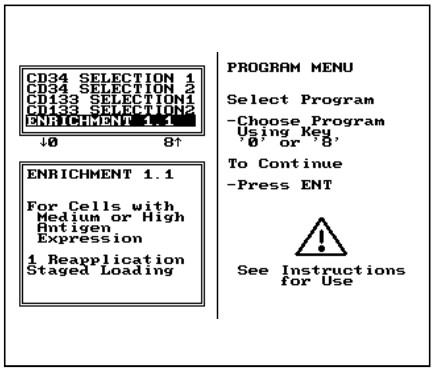
Select the tubing set by highlighting it in the box at the top left hand side with the black bar. Move the bar up and down by using the keys '0' and '8'.

The window below displays the relevant data such as capacity and reference number (REF) of the selected tubing set.

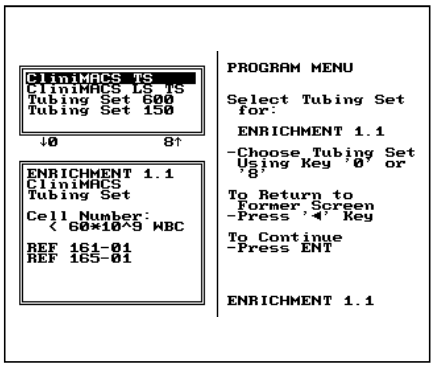
To continue with the selected combination of separation program and tubing set, press ENT

Important

- The separation program ENRICHMENT 1.1 must only be used with the CliniMACS Tubing Set TS (REF 161-01) or the CliniMACS Tubing Set LS (REF 162-01).
- Screen prompts and diagrams serving as procedure guides will appear in the display window. Perform and check each step according to the manual instructions before proceeding to the next step.



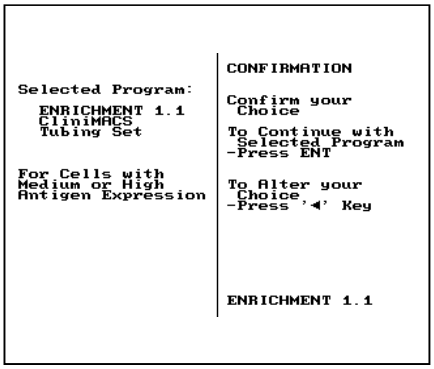
Screen no. 4.2-1



Screen no. 4.2-2

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Confirmation

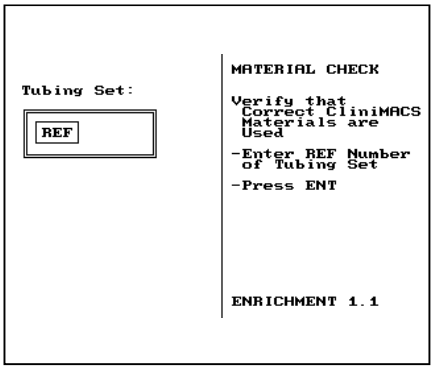


Screen no. 4.2-3

The window will display screen no. 4.2-3 as shown.

To confirm the combination of separation program and tubing set and to proceed, press ENT

Material check



Screen no. 4.2-4

The window will show screen no. 4.2-4 as shown.

The query of the reference number of the tubing set serves as a security check to ensure that the tubing set the user selected is used.

Enter reference number of the selected tubing set and proceed with pressing ENT

If a non-corresponding reference number has been entered, a message appears. To confirm press 'ENT' and enter the correct reference number. If the entered reference number is still wrong, the message appears a second time. After pressing 'ENT' the program will return to the program menu (see screen no. 4.2-2).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Sample parameter input

If the material check was successful, the program continues automatically with the query for some sample parameters that are necessary to adjust the separation to each individual sample and to provide the user with important information regarding required buffer and bag volumes.

Screen no. 4.2-5 will appear as shown.

1. Start by entering the WBC concentration of the sample. The allowed range is shown in the box on the bottom left hand side. Press the "Undo" key to correct a wrong input.

   Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see note).

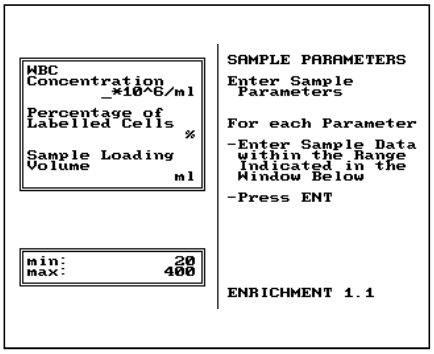
   To continue with the next input, press ENT

2. Enter the percentage of labeled cells. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see note).

   To continue with the next input, press ENT

3. Enter the final volume of the cell sample. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see note).

   To continue, press ENT

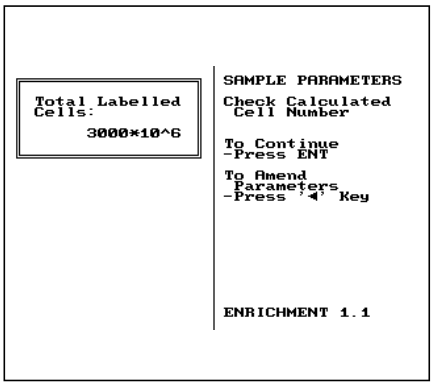


Screen no. 4.2-5

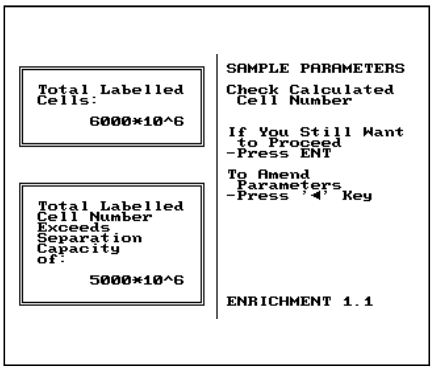
Note

- All sample parameters required refer to the sample post cell labeling and washing procedure.
- The allowed range shown in the box on the bottom left hand side always takes account of the previous input. After each input the software calculates and adjusts the accepted range of the next query to ensure the capacity of the system will not be exceeded.
- **Specifying wrong parameters may result in non-optimal sample processing and may increase the risk of target cell loss.** Entering values for WBC concentration and/or percentage of labeled cells which are lower than the actual sample values may yield in cell loss due to the system being overloaded. Entering a lower sample volume may result in cell loss due to overloading, or even to incomplete sample processing. Specification of any of the three parameters which are too high will yield in increased processing time and enlarged volume of the target cell fraction.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.2-6



Screen no. 4.2-7

Calculation

The CliniMACS Software calculates the total number of labeled cells in order to check whether the separation capacity is sufficient.

The result is shown on one of two possible screens.

- The result of the calculation is shown on a screen similar to screen no. 4.2-6. Ensure the calculated number of labeled cells is correct

  To continue, press ENT

  If the calculated number of labeled cells is not correct, a correction of the data input is possible. Press the "Undo" key to return to the previous screen no. 4.2-5 for correction.

- If the calculated number of labeled cells exceeds the separation capacity of the CliniMACS System a screen similar to screen no. 4.2-7 is shown. Ensure the correct sample parameters have been entered and the calculated number of cells is correct. Press the "Undo" key to return to the previous screen no. 4.2-5 and to correct the sample parameters.

  If the risk of cell loss due to overloading of the system or the increased buffer need is acceptable, proceed with the separation of this sample.

  To proceed with this sample, press ENT

  To prevent overloading the system, split the sample and determine the volume of each portion. Return to the previous screen no. 4.2-5 by pressing the "Undo" key. Enter the sample parameters for the first aliquot of the sample and continue with the separation of this portion. After the separation has been finished, start a second separation with the second aliquot using a new tubing set.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Volume information

From the total number of labeled cells (see previous page) the software calculates the number of separation stages, the amount of buffer needed for the entire separation and the liquid volumes that will be collected in Negative Fraction Bag, Buffer Waste Bag and Cell Collection Bag.

Standard values are the following:

- CliniMACS PBS/EDTA Buffer 1000 mL,
- Negative Fraction Bag 500 mL,
- Buffer Waste Bag 500 mL,
- Cell Collection Bag 150 mL.

If one of the calculated values exceeds these standard values, screen no. 4.2-8 appears to inform the user regarding the amount of liquid that will be required and accumulated during the separation process. Replace the bags delivered with the tubing set (capacity approximately 500 mL) with alternative appropriate size bags, if replacement is necessary. The bags originally attached to the tubing set are connected by Luer connections. Bags that replace the original bags should have a female luer connector. Standard bags can be connected by a Luer/Spike Interconnector.

1. Confirm the requested amount of buffer is available. Do not attach more than 3 liters of buffer on the bag hanger. The bag hangers are designed for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.

2. Open the tray of the tubing set under sterile conditions and replace the original bags with larger ones if necessary. Ensure unrestricted flow to these bags.

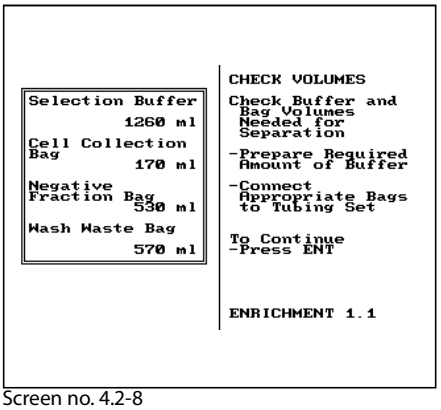
3. Attach a Cell Collection Bag of sufficient size to the free Luer connector. If not specified on screen no. 4.2-8, the maximum elution volume of the target cell fraction is 150 mL. Confirm that unrestricted flow to the Cell Collection Bag is possible.

4. Check luer lock connections on the columns. Luer locks must be closed tightly.

To continue, press ENT

The program automatically continues with the instructions to install the tubing set on the instrument.

Proceed to STEP 3.



Screen no. 4.2-8

Important

- Ensure the required amount of buffer is available and the volume of the bags described on screen no. 4.2-8 is sufficient. Otherwise the performance of the separation will be compromised.

- The bag hangers are designed for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.

- Any modifications of the CliniMACS Tubing Sets should be performed under sterile conditions, e.g. in the laminar flow hood.

- For further analysis or cell processing please note that the volumes listed on screen No. 4.2-8 are not the exact volumes but volumes within the safety requirements.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 2:

ENRICHMENT 3.2

Switch-on of the CliniMACS® plus Instrument

Switch on the CliniMACS® plus Instrument by using the ON/OFF switch located on the back panel of the instrument. Record the date and start time of the instrument run.

The window will display screens no. 1.1 and no. 1.2 as shown in chapter 1.3.9.

To proceed to the program menu, press ENT

Important

- The separation program ENRICHMENT 3.2 must only be used with the CliniMACS Tubing Set TS (REF 161-01).
- Screen prompts and diagrams serving as procedure guides will appear in the display window. Perform and check each step according to the manual instructions before proceeding to the next step.

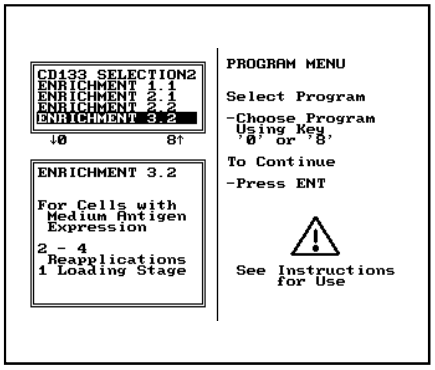
Choice of separation program ENRICHMENT 3.2

The window will display screen no. 4.2-1 as shown.

Choose ENRICHMENT 3.2.

To choose the separation program, highlight the name of the program with the black bar. Move the bar up and down by using the keys '0' and '8'.

To proceed with the highlighted program, press ENT



Screen no. 4.2-1

Selection of tubing set

The window will display screen no. 4.2-2 as shown.

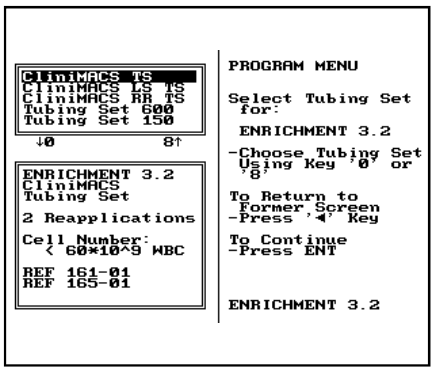
Confirm the correct program has been chosen.

If not, press the "Undo" key (see fig. 1.16) to return to the previous step in order to amend the choice.

Select the tubing set by highlighting it in the box on the left hand side with the black bar. Move the bar up and down using the keys '0' and '8'.

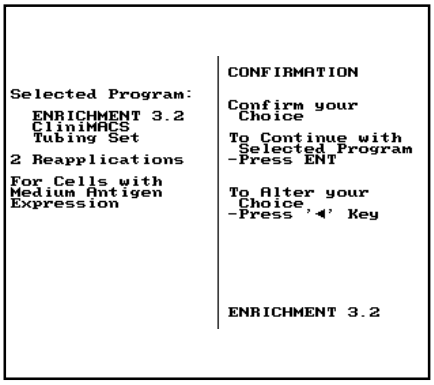
The window below displays the relevant data such as capacity and reference number (REF) of the selected tubing set.

To continue with the selected combination of separation program and tubing set, press ENT



Screen no. 4.2-2

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.2-3

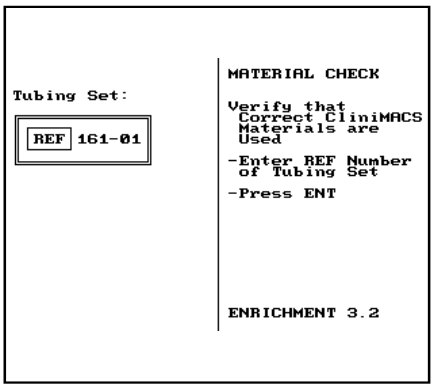
Confirmation of program choice

The window will display screen no. 4.2-3 as shown.

Confirm the correct program has been chosen.

If not, press "Undo" key (see fig. 1.16) to return to the previous step in order to amend the choice.

To confirm and proceed, press ENT



Screen no. 4.2-4

Material check

The window will show screen no. 4.2-4 as shown.

The query of the reference number of the tubing set serves as a security check to ensure that the tubing set the user selected is used.

Enter the reference number of the tubing set and proceed with pressing ENT

If a non-corresponding reference number has been entered, a message appears.

To confirm and proceed, press 'ENT' and enter the correct reference number. If the entered reference number is still wrong, the message appears a second time. After pressing 'ENT' the program will return to the program menu (see screen no. 4.2-2).

Proceed to STEP 3.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 2:

DEPLETION 2.1

Switch-on of the CliniMACS® plus Instrument

Switch on the CliniMACS® plus Instrument by using the ON/OFF switch located on the back panel of the instrument.

The window will display screens no. 1.1 and no. 1.2 as shown in chapter 1.3.9.

To proceed to the program menu, press ENT

Choice of separation program DEPLETION 2.1

The window will display screen no. 4.2-1 as shown.

Choose DEPLETION 2.1.

To choose the separation program, highlight the name of the program with the black bar. Move the bar up and down by using the keys '0' and '8'.

To proceed with the highlighted program, press ENT

Selection of the tubing set

The window will display screen no. 4.2-2 as shown.

Confirm that the correct program has been chosen.

If not, press the "Undo" key (see, Fig. 1.16) to return to the previous step in order to amend the choice.

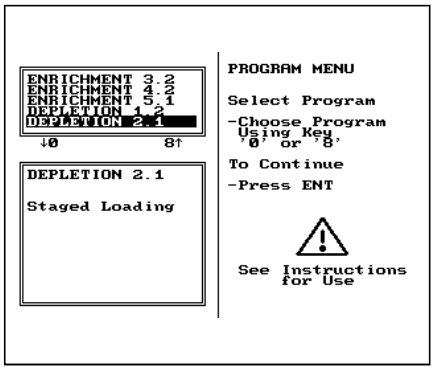
Select the tubing set by highlighting it in the box at the top left hand side with the black bar. Move the bar up and down by using the keys '0' and '8'.

The window below displays the relevant data such as capacity and reference number (REF) of the selected tubing set.

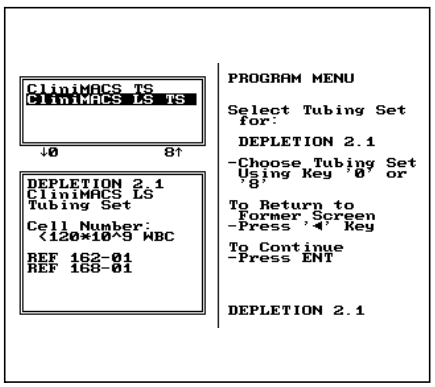
To continue with the selected combination of separation program and tubing set, press ENT

Important

- The separation program DEPLETION 2.1 must only be used with the CliniMACS Tubing Set LS (REF 162-01).
- Screen prompts and diagrams serving as procedure guides will appear in the display window. Perform and check each step according to the manual instructions before proceeding to the next step.



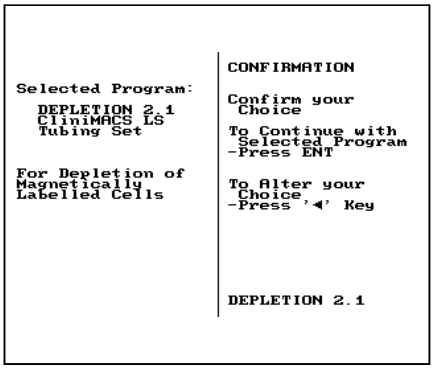
Screen no. 4.2-1



Screen no. 4.2-2

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Confirmation

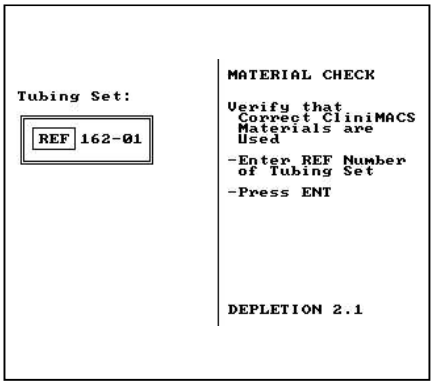


Screen no. 4.2-3

The window will display screen no. 4.2-3 as shown.

To confirm the combination of separation program and tubing set and to proceed, press ENT

Material check



Screen no. 4.2-4

The window will show screen no. 4.2-4 as shown.

The query of the reference number of the tubing set serves as a security check to ensure that the tubing set the user selected is used.

Enter reference number of the selected tubing set and proceed with pressing ENT

If a non-corresponding reference number has been entered, a message appears. To confirm press 'ENT' and enter the correct reference number. If the entered reference number is still wrong, the message appears a second time. After pressing 'ENT' the program will return to the program menu (see screen no. 4.2-2).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Sample parameter input

If the material check was successful, the program continues automatically with the query for different sample parameters that are necessary to adjust the separation to each individual sample and to provide the user with important information regarding required buffer and bag volumes.

Screen no. 4.2-5 will appear as shown.

1. Start by entering the WBC concentration of the sample. The acceptable range is shown in the box on the bottom left hand side. Press the "Undo" key to correct a wrong input.

   Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see note).

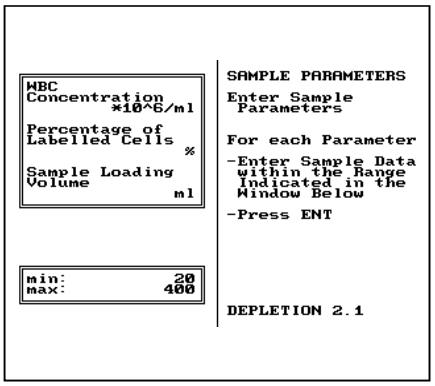
   To continue with the next input, press ENT

2. Enter the percentage of labeled cells. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see note).

   To continue with the next input, press ENT

3. Enter the final volume of the cell sample. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see note).

   To continue, press ENT



Screen no. 4.2-5

Important

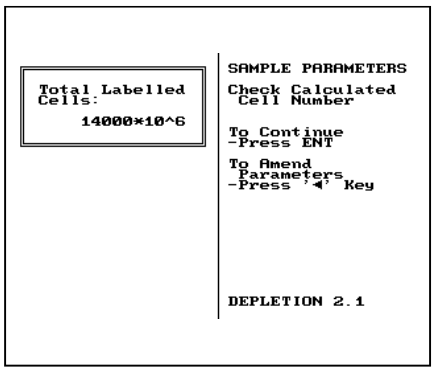
- All sample parameters required refer to the sample post cell labeling and washing procedure.
- The acceptable range shown in the box on the bottom left hand side always takes account of the previous input. After each input the software calculates and adjusts the accepted range of the next query to ensure the separation capacity of the system will not be exceeded.
- Specifying wrong parameters may result in non-optimal sample processing and may increase the risk of target cell loss. Entering values for WBC concentration and/or percentage of labeled cells which are lower than the actual sample values may yield in cell loss due to the system being overloaded. Entering a lower sample volume may result in cell loss due to overloading or even to incomplete sample processing. If the specification of any of the three parameters is too high, this will result in increased processing time and enlarged volume of the target cell fraction.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Calculation

The CliniMACS Software calculates the total number of labeled cells in order to check whether the separation capacity is sufficient.

The result is shown on one of two possible screens.

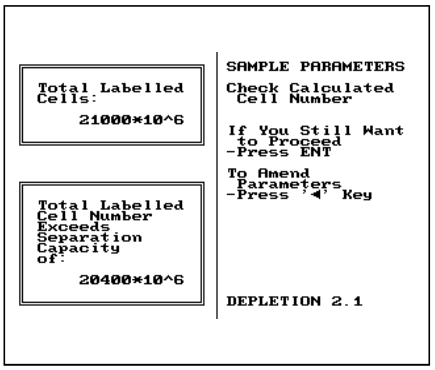


Screen no. 4.2-6

- The result of the calculation is shown on a screen similar to screen no. 4.2-6. Ensure the calculated number of labeled cells is correct.

  To continue, press ENT

  If the calculated number of labeled cells is not correct, a correction of the data input is possible. Press the "Undo" key to return to the previous screen no. 4.2-5 for correction.



Screen no. 4.2-7

- If the calculated number of labeled cells exceeds the separation capacity of the CliniMACS System a screen similar to screen No. 4.2-7 is shown. Ensure the correct sample parameters have been entered and the calculated number of cells is correct. Press the "Undo" key to return to the previous screen No. 4.2-5 and to correct the sample parameters.

  Consider that depletion conditions outside the specifications for tubing set and separation program result in lower depletion efficiency and target cell recovery.

  To prevent overloading the system, split the sample and determine the volume of each portion. Return to the previous screen No. 4.2-5 by pressing the "Undo" key. Enter the sample parameters for the first aliquot of the sample and continue with the separation of this portion. After the separation has been finished, start a second separation with the second aliquot using a new tubing set.

  To proceed with this sample, press ENT

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Volume information

From the total number of labeled cells (see previous page) the software calculates the number of separation stages, the amount of buffer needed for the entire separation and the liquid volumes that will be collected in the Priming Waste Bag, Cell Collection Bag, Negative Fraction Bag and Buffer Waste Bag.

Standard values are the following:

- CliniMACS PBS/EDTA Buffer 1000 mL,
- Negative Fraction Bag 500 mL,
- Buffer Waste Bag 500 mL,
- Cell Collection Bag 160 mL,
- Priming Waste Bag 500 mL.

If one of the calculated values exceeds these standard values, screen No. 4.2-8 appears to inform the user regarding the amount of liquid that will be required and accumulated during the separation process. Replace the bags delivered with the tubing set (capacity approximately 500 mL) with alternative bags of the appropriate size, if replacement is necessary. The bags originally attached to the tubing set are connected by Luer connections. Bags that replace the original bags should have a female luer connector. Standard bags can be connected by a Luer/Spike Interconnector.

1. Confirm the requested amount of buffer is available. Do not attach more than 3 liters of buffer on the bag hanger. The bag hangers are designed for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.

2. Open the tray of the tubing set under sterile conditions and replace the original bags with larger ones if necessary. Ensure unrestricted flow to these bags.

3. Attach a Cell Collection Bag of sufficient size to the free Luer connector. Confirm that unrestricted flow to the Cell Collection Bag is possible.

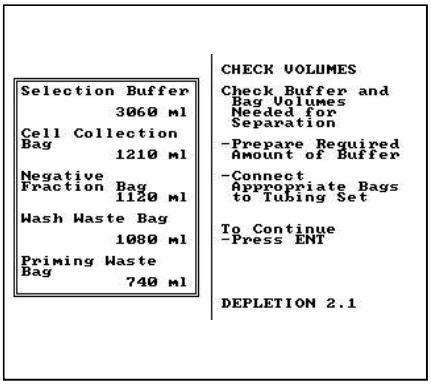
4. Check luer lock connections on the columns. Luer locks must be closed tightly.

5. If the replacement of the Priming Waste Bag is necessary, use the TSCD to replace the bag with a transfer bag of sufficient size.

To continue, press ENT

The program automatically continues with the installation instructions to install the tubing set on the instrument.

Proceed to STEP 3.



Screen no. 4.2-8

Important

- Ensure the required amount of buffer is available and the volume of the bags mentioned on screen no. 4.2-8 is sufficient. Otherwise the performance of the depletion will be compromised.
- The bag hangers are designed for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.
- Any modifications of the CliniMACS Tubing Sets should be performed under sterile conditions, e.g. in the laminar flow hood.
- For further analysis or cell processing please note that the volumes listed on screen no. 4.2-8 are not the exact volumes but volumes within the safety requirements.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

STEP 2:

DEPLETION 3.1

Switch-on of the CliniMACS® plus Instrument

Switch on the CliniMACS® plus Instrument by using the ON/OFF switch located on the back panel of the instrument.

The window will display screens no. 1.1 and no. 1.2 as shown in chapter 1.3.9.

To proceed to the program menu, press ENT

Choice of separation program DEPLETION 3.1

The window will display screen no. 4.2-1 as shown.

Choose DEPLETION 3.1.

To choose the separation program, highlight the name of the program with the black bar. Move the bar up and down by using the keys '0' and '8'.

To proceed with the highlighted program, press ENT

Selection of the tubing set

The window will display screen no. 4.2-2 as shown.

Confirm that the correct program has been chosen.

If not, press the "Undo" key (see fig. 1.16) to return to the previous step in order to amend the choice.

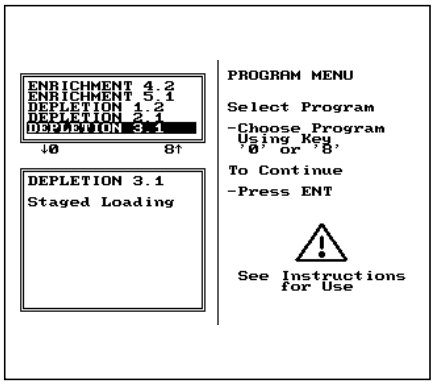
Select the tubing set by highlighting it in the box at the top left hand side with the black bar. Move the bar up and down by using the keys '0' and '8'.

The window below displays the relevant data like capacity and reference number (REF) of the selected tubing set.

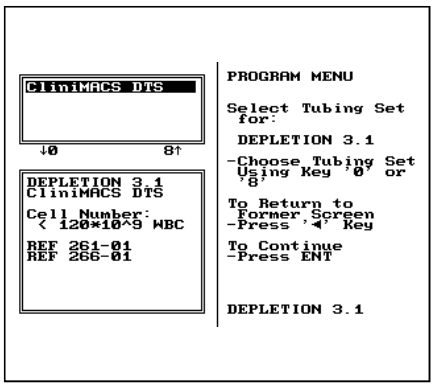
To continue with the selected combination of separation program and tubing set, press ENT

Important

- The separation program DEPLETION 3.1 must only be used with the CliniMACS Depletion Tubing Set (REF 261-01).
- Screen prompts and diagrams serving as procedure guides will appear in the display window. Perform and check each step according to the manual instructions before proceeding to the next step.

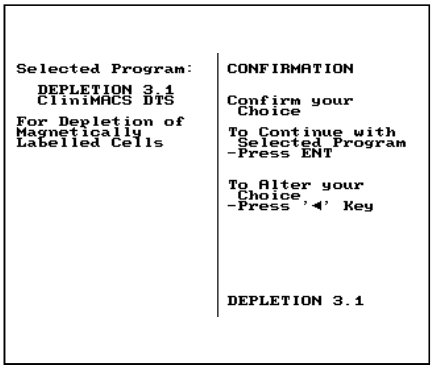


Screen no. 4.2-1



Screen no. 4.2-2

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

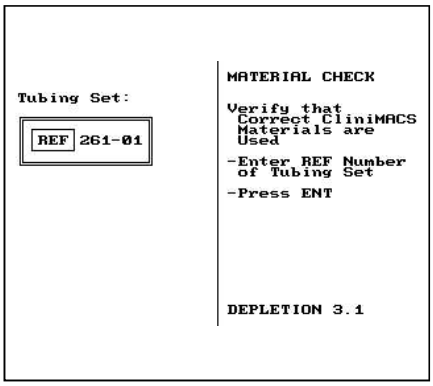


Screen no. 4.2-3

Confirmation

The window will display screen no. 4.2-3 as shown.

To confirm the combination of separation program and tubing set and to proceed, press ENT



Screen no. 4.2-4

Material check

The window will show screen no. 4.2-4 as shown.

The query of the reference number of the tubing set serves as a security check to ensure that the tubing set the user selected is used.

Enter the reference number of the selected tubing set and proceed with pressing ENT

If a non-corresponding reference number has been entered, a message appears. To confirm press 'ENT' and enter the correct reference number. If the entered reference number is still wrong, the message appears a second time. After pressing 'ENT' the program will return to the program menu (see screen no. 4.2-2).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Sample parameter input

If the material check was successful, the program continues automatically with the query for different sample parameters that are necessary to adjust the separation to each individual sample and to provide the user with important information regarding required buffer and bag volumes.

Screen no. 4.2-5 will appear as shown.

1. Start by entering the WBC concentration of the sample. The acceptable range is shown in the box on the bottom left hand side. Press the "Undo" key to correct a wrong input.

   Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see "Important").

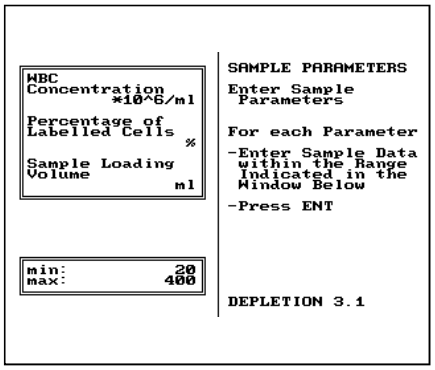
   To continue with the next input, press ENT

2. Enter the percentage of labeled cells. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see note).

   To continue with the next input, press ENT

3. Enter the final volume of the cell sample. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see note).

   To continue, press ENT



Screen no. 4.2-5

Important

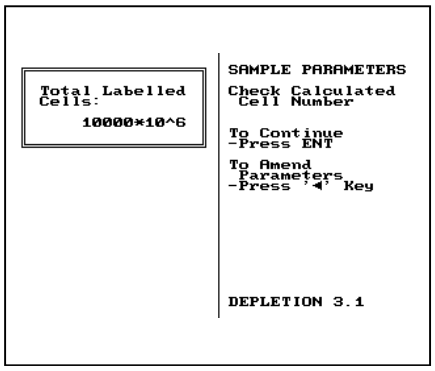
- All sample parameters required refer to the sample **post** cell labeling and washing procedure.
- The acceptable range shown in the box on the bottom left hand side always takes account of the previous input. After each input the software calculates and adjusts the accepted range of the next query to ensure the separation capacity of the system will not be exceeded.
- **Specifying wrong parameters may result in non-optimal sample processing and may increase the risk of lower depletion efficiency.** Entering values for WBC concentration and/or percentage of labeled cells which are lower than the actual sample values may result in lower depletion efficiency due to the system being overloaded. Entering a lower sample volume may result in cell loss due to overloading or even to incomplete sample processing. If the specification of any of the three parameters is too high, processing time will increase and the volume of the target cell fraction will enlarge.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Calculation

The CliniMACS Software calculates the total number of labeled cells in order to check whether the separation capacity is sufficient.

The result is shown on one of two possible screens.

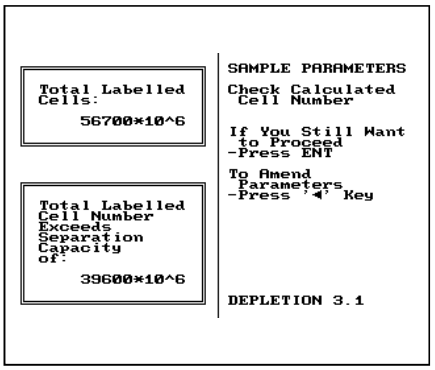


Screen no. 4.2-6

- The result of the calculation is shown on a screen similar to screen no. 4.2-6. Ensure the calculated number of labeled cells is correct.

  To continue, press ENT

  If the calculated number of labeled cells is not correct, a correction of the data input is possible. Press the "Undo" key to return to the previous screen no. 4.2-5 for correction.



Screen no. 4.2-7

- If the calculated number of labeled cells exceeds the separation capacity of the CliniMACS System a screen similar to screen no. 4.2-7 is shown. Ensure the correct sample parameters have been entered and the calculated number of cells is correct. Press the "Undo" key to return to the previous screen no. 4.2-5 and to correct the sample parameters.

  Consider that depletion conditions outside the specifications for tubing set and separation program result in lower depletion efficiency.

  To prevent overloading the system, split the sample and determine the volume of each portion. Return to the previous screen no. 4.2-5 by pressing the "Undo" key. Enter the sample parameters for the first aliquot of the sample and continue with the separation of this portion. After the separation has been finished, start a second separation with the second aliquot using a new tubing set.

  To proceed with this sample, press ENT

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Volume information

From the total number of labeled cells (see previous page) the software calculates the number of separation stages, the amount of buffer needed for the entire depletion and the liquid volumes that will be collected in the Reapplication Bag, Cell Collection Bag, Non-Target Cell Bag and Buffer Waste Bag.

If one of the calculated values exceeds the standard values, a screen similar to no. 4.2-8 appears to inform the user regarding the amount of liquid that will be required and accumulated during the separation process. Replace the bags delivered with the tubing set with alternative bags of the appropriate size, if replacement is necessary. The bags originally attached to the tubing set are connected by Luer connections. Bags that replace the original bags should have a female Luer connector. Standard bags can be connected by a Luer/Spike Interconnector.

1. Confirm the requested amount of buffer is available. Do not attach more than 3 liters of buffer on the bag hanger. The bag hangers are designed for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.

2. Open the tray of the tubing set under sterile conditions and replace the original bags by larger ones or attach aditional bags if necessary. Ensure unrestricted flow to these bags.

3. If replacement is neccessary, replace the Cell Collection Bag of the tubing set with a transfer bag of sufficient size. Determine the weight of the new, empty Cell Collection Bag and record it. Confirm that unrestricted flow to the Cell Collection Bag is possible. Alternatively, attach an additional 600 mL transfer bag to the first Cell Collection Bag, using a plasma transfer set.

4. Check luer lock connections on the columns. Luer locks must be closed tightly.

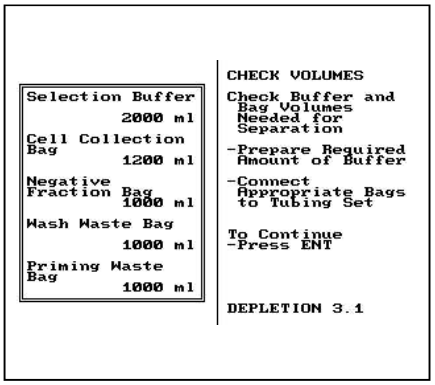
To continue, press ENT

The program continues with the installation instructions to install the tubing set on the instrument.

Proceed to STEP 3.

Important

- Negative Fraction Bag corresponds to Non-Target Cell Bag.
- Priming Waste Bag corresponds to Reapplication Bag.



Screen no. 4.2-8

Important

- Ensure the required amount of buffer is available and the volume of the bags mentioned on screen no. 4.2-8 is sufficient. Otherwise the performance of the separation will be compromised.
- The bag hangers are designed for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.
- Any modifications of the CliniMACS Tubing Sets should be performed under sterile conditions, e.g. in the laminar flow hood.
- For further analysis or cell processing please note that the volumes listed on screen no. 4.2-8 are not the exact volumes but maximal volumes.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

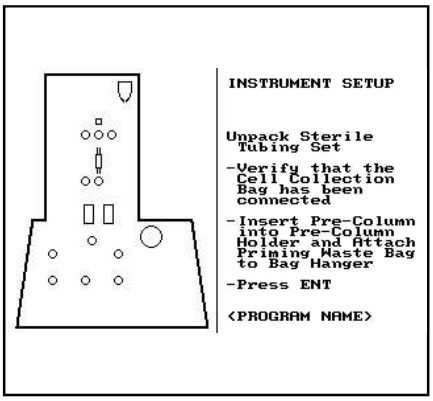
InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 3:

CliniMACS® Tubing Set TS and CliniMACS Tubing Set LS

Preparation for tubing set installation

The window will display screen no. 4.3-1 as shown.



Screen no. 4.3-1

Instructions are provided on the right side of the screen, and a diagram corresponding to the instruction is displayed on the left. The blinking features on the screen indicate the areas requiring attention.

The CliniMACS® Tubing Set TS and the CliniMACS Tubing Set LS are provided in a sealed, sterilized packages. Each tubing set contains preassembled tubing and columns for one cell separation (see fig. 4.3-2). When the packaging is intact, a sterile fluid path is provided.

1. Record the lot number and use-by date of the tubing set. Unpack the sterile tubing set under sterile conditions (e.g. laminar flow hood). Remove the pinch clamp used for packaging of the CliniMACS Tubing Set LS before usage of the tubing set. The clamp is labeled with "Remove before assembly to CliniMACS® plus Instrument".

2. Check luer lock connections to bags. Luer lock must be closed tightly.

Attach Cell Collection Bag

1. Record the weight of the empty Cell Collection Bag.

2. In an aseptic environment, remove caps and attach the sterile Cell Collection Bag to the luer connector on the tubing set before loading the tubing set onto the CliniMACS plus Instrument.

3. Confirm that unrestricted flow to the Cell Collection Bag is possible.

4. Proceed with the installation of the tubing set using either the clean room procedure described below, or the "Alternative installation for the CliniMACS Tubing Sets".

Note

- The CliniMACS plus Instrument shows the chosen program name, e.g. CD34 SELECTION 1, on the bottom line of the instrument screen.

- At any step during the tubing set installation the Undo Key (see fig. 1.16) can be pushed to return to the previous step.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

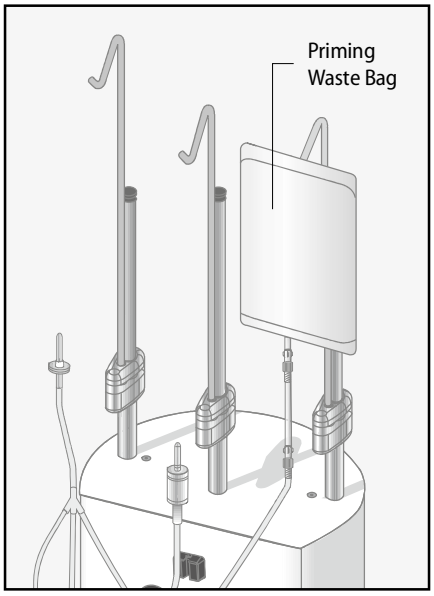


Fig. 4.3-1: Attach Priming Waste Bag to bag hanger.

Attach Priming Waste Bag and insert pre-column

The window will display screen no. 4.3-1 as shown.

1. Attach the Priming Waste Bag to the right hand bag hanger on the instrument as shown (see fig. 4.3-1).

2. Place the pre-column into the holder as shown (see fig. 4.3-5). When the pre-column is placed into the pre-column holder, ensure that the plastic projections found at the bottom of the column are facing the instrument user.

3. Adjust the height of the buffer bag hanger. Raise or lower the hanger to accommodate the size of the Priming Waste Bag. Ensure that it is positioned high enough to prevent severe bending of the tubing that could restrict the flow of liquid, and that it is low enough to avoid the tubing or connections being stretched.

   To proceed, press ENT

Note

- The bag hangers are designed for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.

- When the pre-column is placed into the pre-column holder, ensure that the plastic projections found at the bottom of the column are facing the instrument user.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

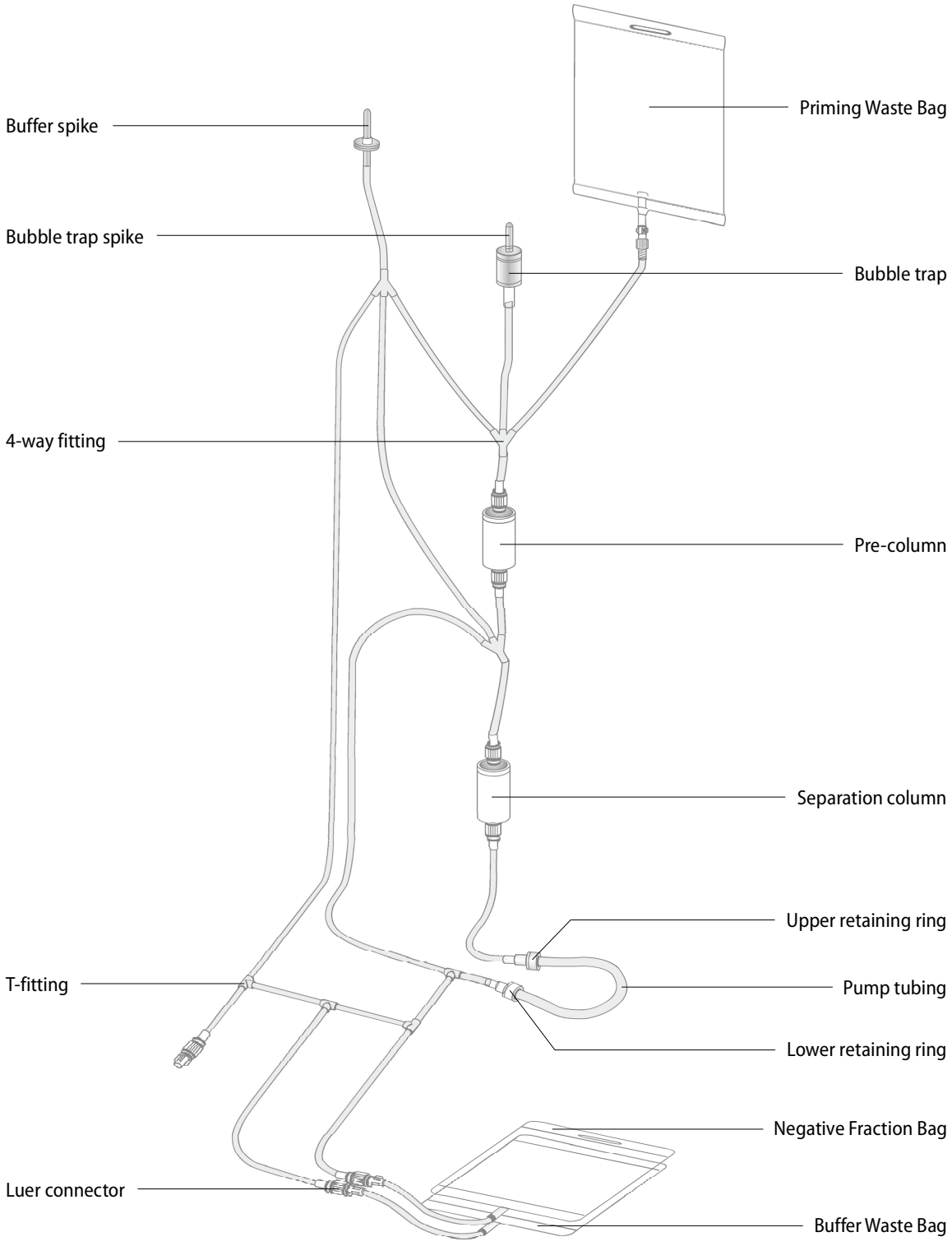
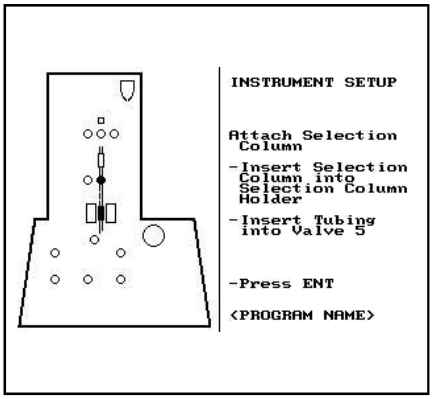


Fig. 4.3-2: General construction of a CliniMACS Tubing Set (e.g. CliniMACS Tubing Set TS, REF 161-10)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.3-2

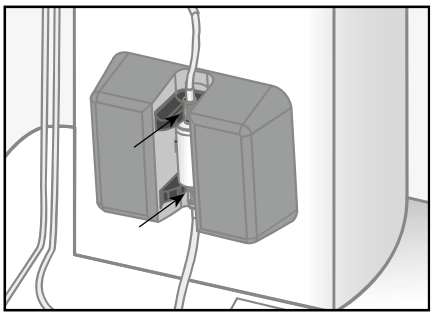


Fig. 4.3-3: Separation column in separation column holder.

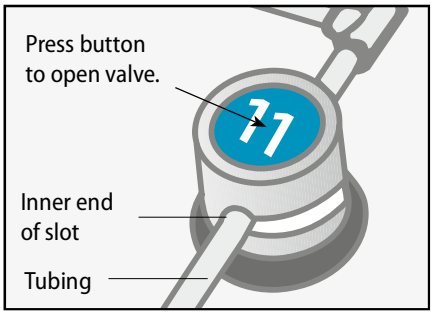


Fig. 4.3-4: Correctly inserted tubing.

Insert separation column and load valve no. 5

The window will display screen no. 4.3-2 as shown.

The valves shown on the screen will be opened automatically.

1. Insert the separation column into the separation column holder as shown (see fig. 4.3-3).

Note

To avoid possible pinch injury, insert the separation column as follows: Hold the top and bottom of the column between thumb and index finger, then carefully insert the separation column into the separation column holder.

2. Load the tubing into valve no. 5.

Note

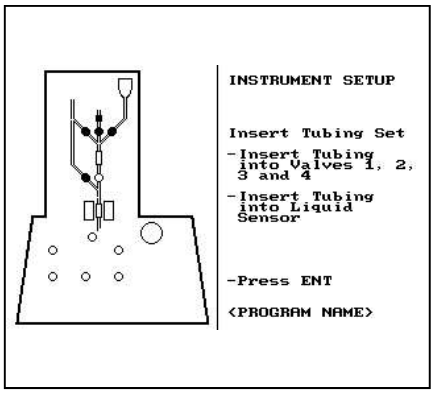
- As each step is performed, check all tubing and attachments for any kinks or severe bending that could restrict the flow of liquid through the tubing. Check all valves to ensure the tubing fits snugly.
- Only insert the tubing set into open valves (when button is pushed inwards). The tubing will not fit correctly if inserted into a closed valve.
- If the tubing has to be adjusted after a valve has been closed, do not pull the tubing without pressing the valve button to open the valve (see fig. 4.3-4).

To proceed, press ENT

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Load valves nos. 1, 2, 3, and 4

The window will display screen no. 4.3-3 as shown.



Screen no. 4.3-3

1. Load the tubing into valve no. 4. Confirm that the tubing is placed securely in the valve opening (see fig. 4.3-4). Pay particular attention to the area between valves nos. 4 and 5 (see fig. 4.3-5).

2. Insert the tubing into valve no. 1.

3. Position the 4-way fitting just below valve no. 2. Pay particular attention to the area below valve no. 2 (see fig. 4.3-5).

4. Insert the tubing into valve nos. 2 and 3.

5. Mount the tubing between valve no. 2 and the bubble trap into the liquid sensor (see fig. 4.3-5). Confirm that the tubing is placed correctly into the sensor fitting.

Note

To assure proper operation, both the liquid sensor and the tubing being inserted **must be dry**. Carefully inspect both. If any liquid is present, dry the area with a soft, lint-free cloth.

To proceed, press ENT

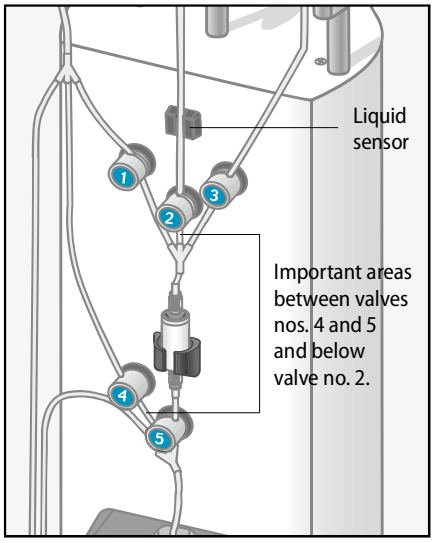
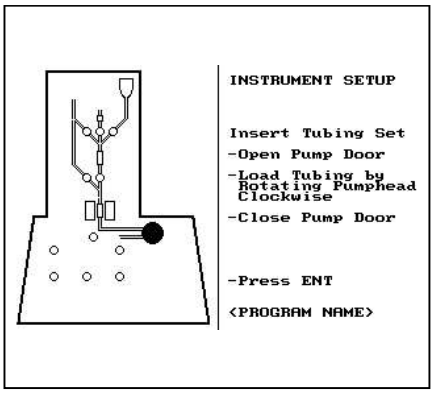


Fig. 4.3-5: Tubing in valves.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.3-4

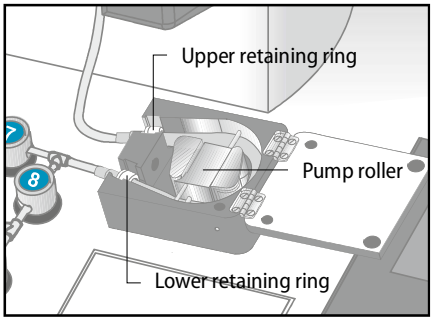


Fig. 4.3-6: Loading of pump tubing.

Load pump tubing

The window will display screen no. 4.3-4 as shown.

1. Open the pump door by lifting up at the left hand edge.
2. Insert the upper retaining ring on the pump tubing into the retaining ring groove on the pump housing (see fig. 4.3-6).
3. Rotate the pump roller clockwise (see fig. 4.3-6) until the tubing is threaded between both sets of the tubing guide pins and the tubing fits snugly around the pump roller. Ensure the tubing is not pinched at the end of the guide pins. If adjustment of the tubing inside the pump is neccessary, the tubing can be unloaded by lifting the lower end and turning the pump roller anti-clockwise.
4. Insert the lower retaining ring on the pump tubing into the retaining ring groove on the pump housing (see fig. 4.3-6).
5. Repeat clockwise rotation of the pump roller, to be certain that the pump roller moves freely.
6. Close the pump door.

Note

During the cell separation program the pump will immediately stop the run whenever the pump housing is opened. If left open for more than 600 seconds the instrument will abort the run in progress.

To proceed, press ENT

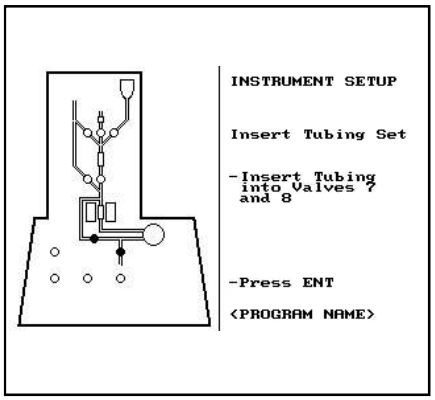
CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Load valves nos. 7 and 8

The window will display screen no. 4.3-5.

1. Load the tubing into valve no. 7.
2. Load the tubing into valve no. 8.

To proceed, press ENT



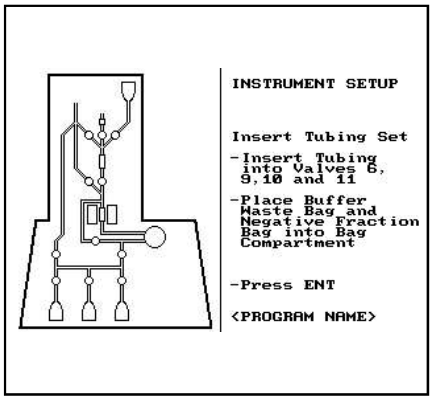
Screen no. 4.3-5

Load valves nos. 6, 9, 10, and 11

The window will display screen no. 4.3-6.

1. Load the tubing into valves nos. 6, 9, 10, and 11.
2. Place the Negative Fraction Bag and the Buffer Waste Bag in the bag compartment. Confirm the tubing is not compressed under the bag compartment lid.

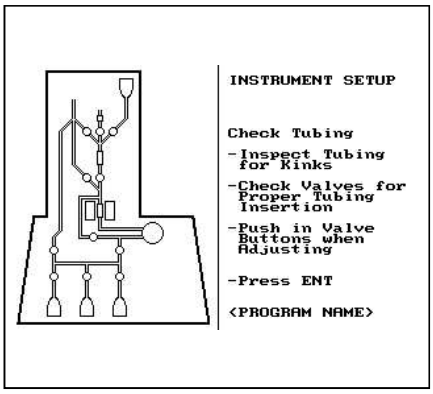
To proceed, press ENT



Screen no. 4.3-6

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Recheck all tubing and attachments



Screen no. 4.3-7

The window will display screen no. 4.3-7.

Before beginning the run, recheck all tubing and attachments.

Note

- Check all valves for proper tubing insertion. Confirm that the tubing is spaced uniformly, and that there are no kinks or stretched areas in the tubing. Pay particular attention to the pre-column area, as well as the area between the pump and valves nos. 7 and 8 (see fig. 4.3-7), and between valves nos. 4 and 5 (see fig. 4.3-5).
- If the tubing must be adjusted after a valve has been closed, do not pull the tubing without pressing the valve button to open the valve. If any tubing has been adjusted, it is absolutely necessary to press the corresponding valves firmly two times.

To proceed, press ENT

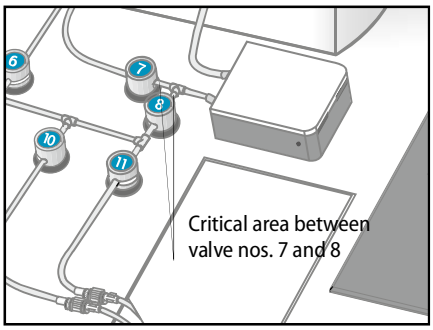
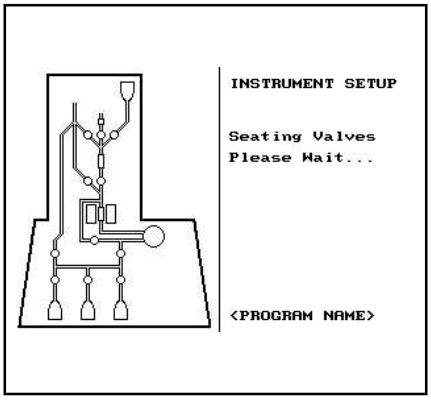


Fig. 4.3-7: Tubing in valves

Seating of valves



Screen no. 4.3-8

The window will display screen no. 4.3-8.

In order to ensure the proper fit of tubing in the valves, the CliniMACS plus Instrument will operate all of the valves in sequence, twice. Watch and listen to confirm that all valves are working properly. If any valve does not operate correctly, refer to the troubleshooting section. This step can be repeated by using the "Undo" Key followed by the Enter Key (see fig. 1.16).

The magnet drive is also be tested during this check sequence.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Attach CliniMACS® PBS/EDTA Buffer

The window will display screen no. 4.3-9.

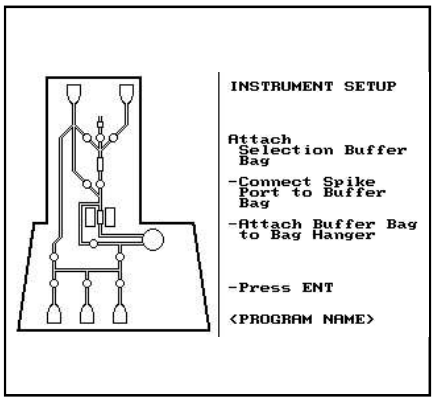
The prescribed buffer for CliniMACS® Separations is CliniMACS PBS/EDTA Buffer supplemented with HSA to a final concentration of 0.5% (w/v).

1. Using aseptic technique, remove the cap from the buffer spike on the tubing set (see fig. 4.3-2) and connect it to the buffer bag. Ensure that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to confirm that the spike has penetrated the bag.

2. Attach the buffer bag to the buffer bag hook on the bag hanger (see fig. 4.3-8).

3. Adjust the height of the buffer bag hanger. Raise or lower the hanger to accommodate the size of the buffer bag. Ensure that it is positioned high enough to prevent severe bending of the tubing that could restrict the flow of fluid, and that it is low enough to avoid the tubing or connections being stretched (see fig. 4.3-8).

To proceed, press ENT



Screen no. 4.3-9

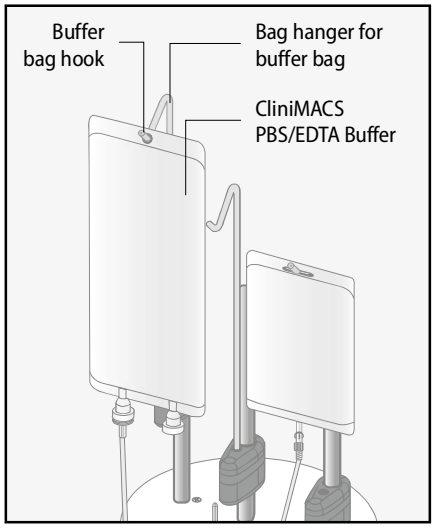


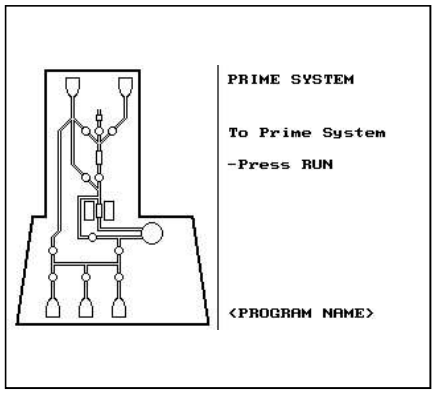
Fig. 4.3-8: Attach CliniMACS PBS/EDTA Buffer to bag hanger.

Note

The bag hangers are designed for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Start priming



Screen no. 4.3-10

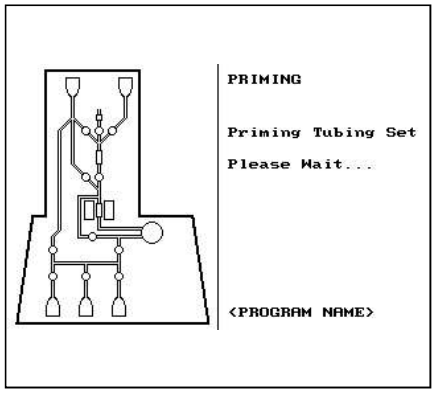
The window will display screen no. 4.3-10.

To start priming, press RUN

The window will display screen no. 4.3-11.

During the priming phase the tubing set is filled with buffer. The buffer will be circulated through the tubing set including both the pre-column and the separation column. Priming waste is collected in the Priming Waste Bag (23) and the Buffer Waste Bag (32, fig. 4.3-2). The priming cycles will continue, repeating a series of steps. The priming phase takes approximately 1 minute. Priming status is updated on the display.

Check during the priming



Screen no. 4.3-11

During the priming phase, check all tubing, fittings, valves, and columns for the appearance of any leaks or the presence of any folds that may block fluid flow.

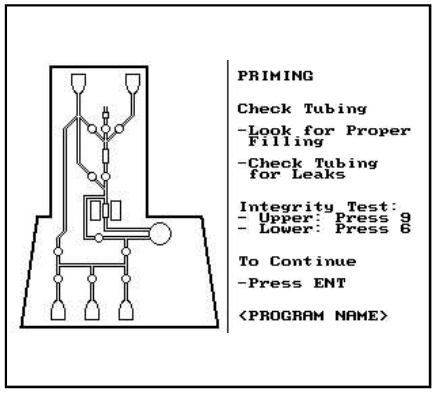
If leaks or malfunctions are observed, stop run by pressing 'STOP'. The operator has 600 seconds to resolve the problem. Restart the process by pressing the 'RUN'.

After 600 seconds, the separation is automatically aborted. If the problem cannot be resolved or if the tubing set is defective remove the tubing set and replace it with a new one. Maintain the tubing set and contact Miltenyi Biotec's Customer Service department for instructions regarding the return of the defective tubing set.

Note

Once priming has started, it is not possible to return to the instrument set-up procedure.

Final check of all tubing and attachments



Screen no. 4.3-12

The window will display screen no. 4.3-12.

Before beginning the run, check the following:

- fluid in all parts of tubing set,
- **no** excess air in tubing set,
- fluid in the Priming Waste Bag and the Buffer Waste Bag,
- **no** fluid in the Negative Fraction Bag or in the Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Integrity test

For additional safety, an integrity test must be performed to test the tubing set for leaks. The test sequence consists of two automated sequences, which allow both the upper and the lower parts of the tubing set to be over pressurized and tested separately.

Integrity test for the upper part of the tubing set

1. When the operator performs "Final check of all tubing and attachments" the window displays screen no. 4.3-12.

2. After performing the "Final check of all tubing and attachments", DO NOT press ENT.

3. To enter the integrity test for the upper part, press [9]

4. The window will display screen no. 4.3-13.

5. To start the test sequence, press [RUN]

   To return to screen no. 4.3-12, press [3]

6. Once the RUN button has been pressed, the instrument starts the automated test sequence for the upper part of the tubing set.

   The window will display screen no. 4.3-14.

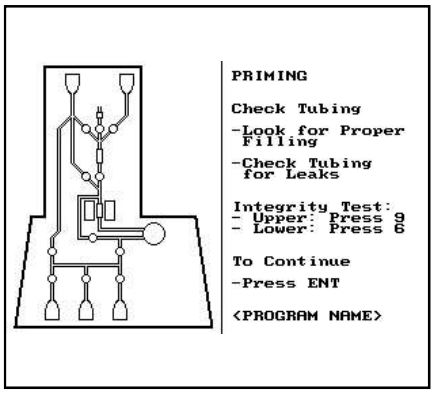
   Overpressure will be created and held for 2 minutes. During this time the operator should watch the connections above and under the pre-column and separation column, and the upper pump tube connection.

   At each point the test sequence can be finished by pressing 'ENT'.

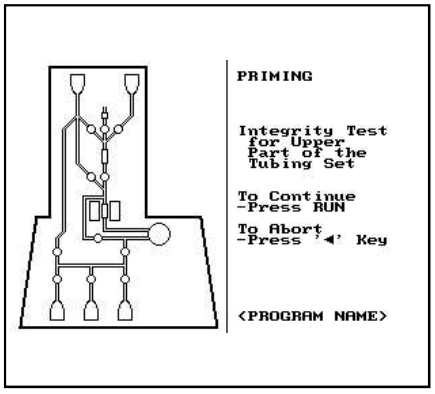
7. After 2 minutes the pressure is automatically released, and the window displays screen no. 4.3-12.

   Using a tissue, the operator should check to determine if any leaks have occurred during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced with a new one. Keep the defective tubing set and contact Miltenyi Biotec's Customer Service department for instructions regarding the return of the tubing set to Miltenyi Biotec.

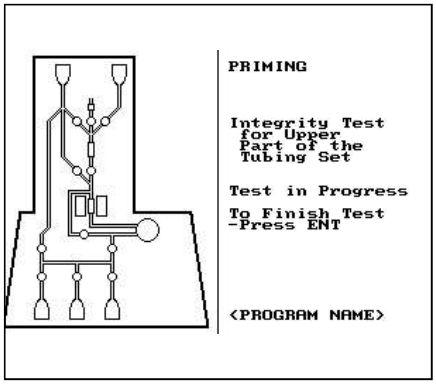
8. If no leaks are observed, continue with the integrity test of the lower part of the tubing set.



Screen no. 4.3-12

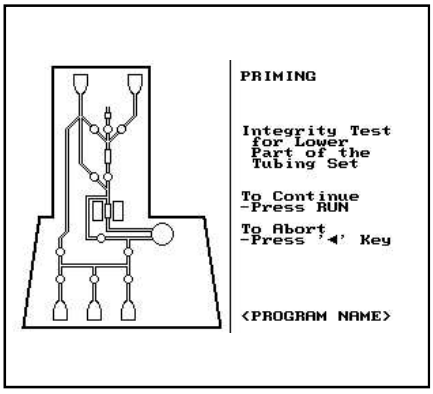


Screen no. 4.3-13

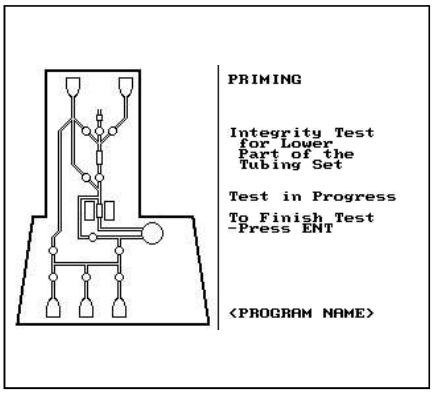


Screen no. 4.3-14

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.3-15



Screen no. 4.3-16

Integrity test for the lower part of the tubing set

1. The window displays screen no. 4.3-12.

2. To enter the integrity testfor the lower part, press 6
   DO NOT press 'ENT'.

3. The window will display screen 4.3-15.

4. To start the test sequence, press RUN

   To go back to screen no. 4.3-12, press 3

5. Once the RUN button has been pressed, the instrument starts the automated test sequence for the lower part of the tubing set.

   The window will display screen no. 4.3-16.

   Overpressure will be created and held for 30 seconds. During this time the operator should watch the lower pump tube connection and the T-fittings between valves nos. 6, 8, 9, 10, and 11.

   At each point the test sequence can be finished by pressing 'ENT'.

6. After 30 seconds the pressure is automatically released, and the window displays screen no. 4.3-12.

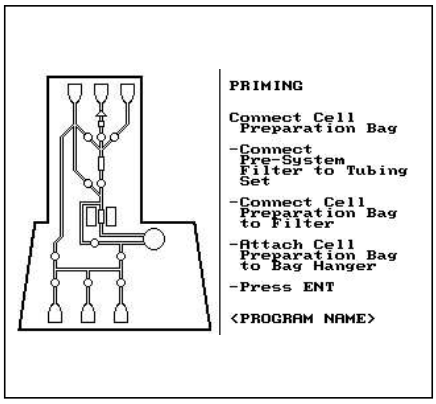
   Using tissue, the operator should now check to determine if any leaks have occurred during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced with a new one. Keep the defective tubing set and contact Miltenyi Biotec's Customer Service department for instructions regarding the return of the tubing set to Miltenyi Biotec.

7. If no leaks are observed the operator can continue with the next step by pressing ENT

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Connect Cell Preparation Bag

The window will display screen no. 4.3-17.



Screen no. 4.3-17

After the priming phase has been completed and no leaks or malfunctions are observed, the Cell Preparation Bag can be attached (see fig. 4.3-9). Use aseptic techniques for all steps.

Connect the Cell Preparation Bag containing the magnetically labeled and washed cells with the pre-system filter:

1. Remove the cap from the bubble trap spike of the bubble trip (see fig. 4.3-2).

2. Remove the cap from the lower opening of the pre-system filter (see fig. 4.3-9). Firmly insert the spike into the pre-system filter. DO NOT remove the top cap of the pre-system filter.

3. Remove the cap from the pre-system filter spike (see fig. 4.3-9).

4. Spike the Cell Preparation Bag with the pre-system filter see fig 4.3-9) ensuring that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to confirm that the spike has penetrated the bag.

5. Check the connection between the pre-system filter and the tubing set to confirm that the connection is secure.

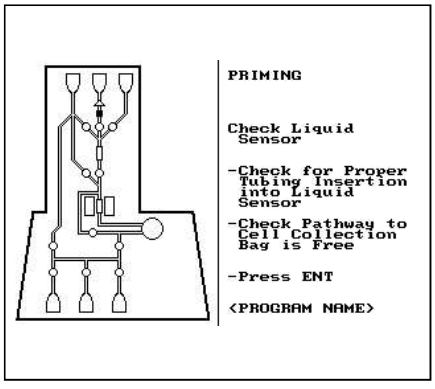
6. Hang the Cell Preparation Bag on the bag hanger.

7. Adjust the bag hanger for the Cell Preparation Bag to hold the Cell Preparation Bag in an upright position.

To proceed, press ENT

Final check of the liquid sensor

The window will display screen no. 4.3-18.



Screen no. 4.3-18

1. Check the liquid sensor tubing. Ensure the tubing has been properly inserted, that it is free of any external liquid and has not been dislodged during the loading procedure.

2. Confirm that unrestricted flow to the Cell Collection Bag is possible.

To proceed, press ENT

Proceed to STEP 4.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

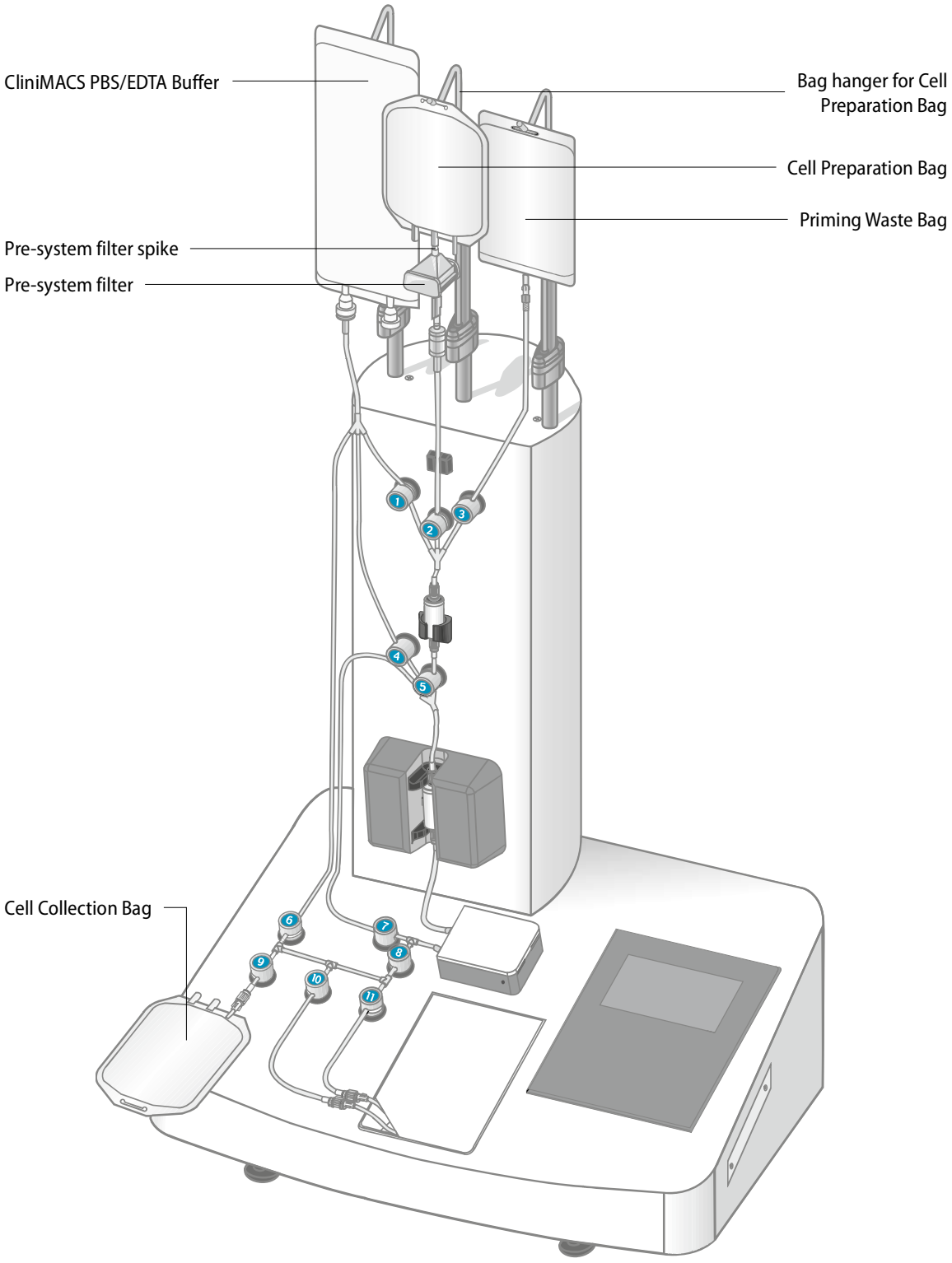


Fig. 4.3-9: CliniMACS plus Instrument with CliniMACS Tubing Set, CliniMACS PBS/EDTA Buffer, Cell Preparation Bag, and Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Alternative installation of CliniMACS® Tubing Sets

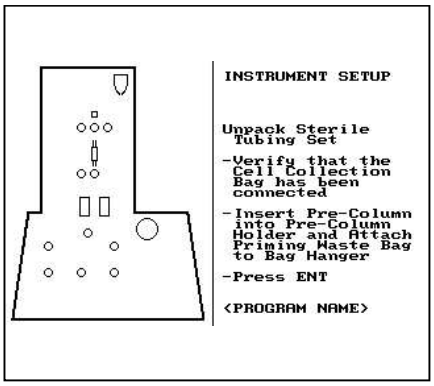
The instructions in STEP 3 and the screens displayed by the CliniMACS® plus Instrument describe the installation of the CliniMACS Tubing Sets under sterile conditions (clean room).

The CliniMACS Plus System itself is a closed system which does not necessarily must be operated in a clean room. However, if operated outside a clean room, the installation procedure of the tubing set needs to be adapted in order to ensure that the sterility of the cell separation process is guaranteed.

The sterility of the cell separation process may be compromised during the attachment of the Cell Collection Bag, the CliniMACS PBS/EDTA Buffer, the pre-system filter, and the Cell Preparation Bag. To ensure that the system remains sterile, these components must be attached to the tubing set under sterile conditions (e.g. laminar flow hood). When the components are attached to the tubing set before installation onto the CliniMACS plus Instrument, the order of the instructions provided by the instrument and the instructions in STEP 3 must be changed and further actions taken.

When operating the CliniMACS plus Instrument outside a clean room, follow the following additional instructions, altering the instructions in STEP 3.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.3-1

Preparation for tubing set installation

The window will display screen no. 4.3-1.

As described, the Cell Collection Bag, the CliniMACS PBS/EDTA Buffer, the pre-system filter and the Cell Preparation Bag must be attached to the CliniMACS Tubing Sets before installing the tubing set onto the instrument under sterile conditions.

Unpack the tubing set under sterile conditions and attach the following components under sterile conditions:

1. **Attachment of Cell Collection Bag**
   Follow the instructions.

Attach Cell Collection Bag

2. **Attachment of CliniMACS PBS/EDTA Buffer**
   Clamp the tubing just below the buffer spike with locking forceps in order to prevent the buffer from flowing into the tubing set during installation (see fig. 4.3-10). Using aseptic technique remove the cap from the buffer spike on the tubing set and connect it to the buffer bag. Ensure that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to ascertain that the spike has penetrated the bag.

3. **Attachment of pre-system filter**
   Remove the cap from the spike of the bubble trap. Remove the cap from the lower opening of the pre-system filter. Firmly insert the spike into the pre-system filter. DO NOT remove the top cap of the pre-system filter. Close the tubing just below the bubble trap using locking forceps (see fig. 4.3-10). This prevents the prepared cell suspension in the Cell Preparation Bag from entering the pre-system filter.

4. **Attachment of Cell Preparation Bag**
   Connect Cell Preparation Bag containing the magnetically labeled and washed cells to the tubing set. Spike the Cell Preparation Bag with the pre-system filter ensuring that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to ascertain that the spike has penetrated the bag.

5. **Installation on the CliniMACS plus Instrument**
   Hang the buffer bag on the left bag hanger, the Cell Preparation Bag on the middle bag hanger and the Priming Waste Bag on the right bag hanger on the instrument.

   To proceed, press ENT

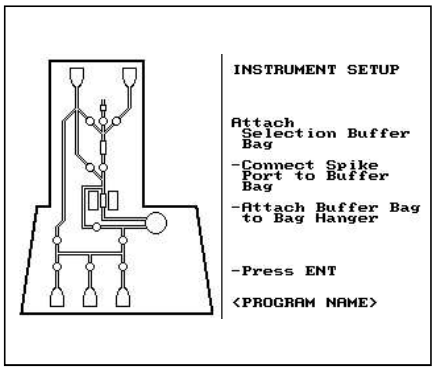
CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Follow the instructions.

- **Attach Priming Waste Bag and insert pre-column**
- **Insert separation column and load valve no. 5**
- **Load valves nos. 1, 2, 3, and 4**
- **Load pump tubing**
- **Load valves nos. 7 and 8**
- **Load valves nos. 6, 9, 10, and 11**
- **Recheck all tubing and attachments**
- **Seating of valves**

Attach CliniMACS® PBS/EDTA Buffer

The window will display screen no. 4.3-9.



Screen no. 4.3-9

1. The buffer bag was attached during "Preparation for tubing set installation". Therefore, only the height of the buffer bag hanger may must be adjusted. Raise or lower the hanger to accommodate the size of the buffer bag, ensuring that the height allotted is high enough to prevent the tubing from severe bending that could restrict the flow of liquid, and low enough to avoid stretching the tubing or connections.

2. Remove the locking forceps from the tubing just below the buffer spike.

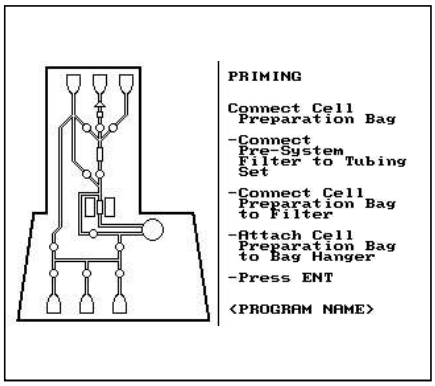
To proceed, press ENT

Follow the instructions.

- **Start priming**
- **Check during priming**
- **Final check of all tubing and attachments**
- **Integrity test**

Connect Cell Preparation Bag (& pre-system filter)

The window will display screen no. 4.3-17.



Screen no. 4.3-17

1. The Cell Preparation Bag and the pre-system filter were attached during "Preparation for tubing set installation".

2. Remove the locking forceps below the bubble trap.

To proceed, press ENT

Follow the instructions.

- **Final check of the liquid sensor**

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

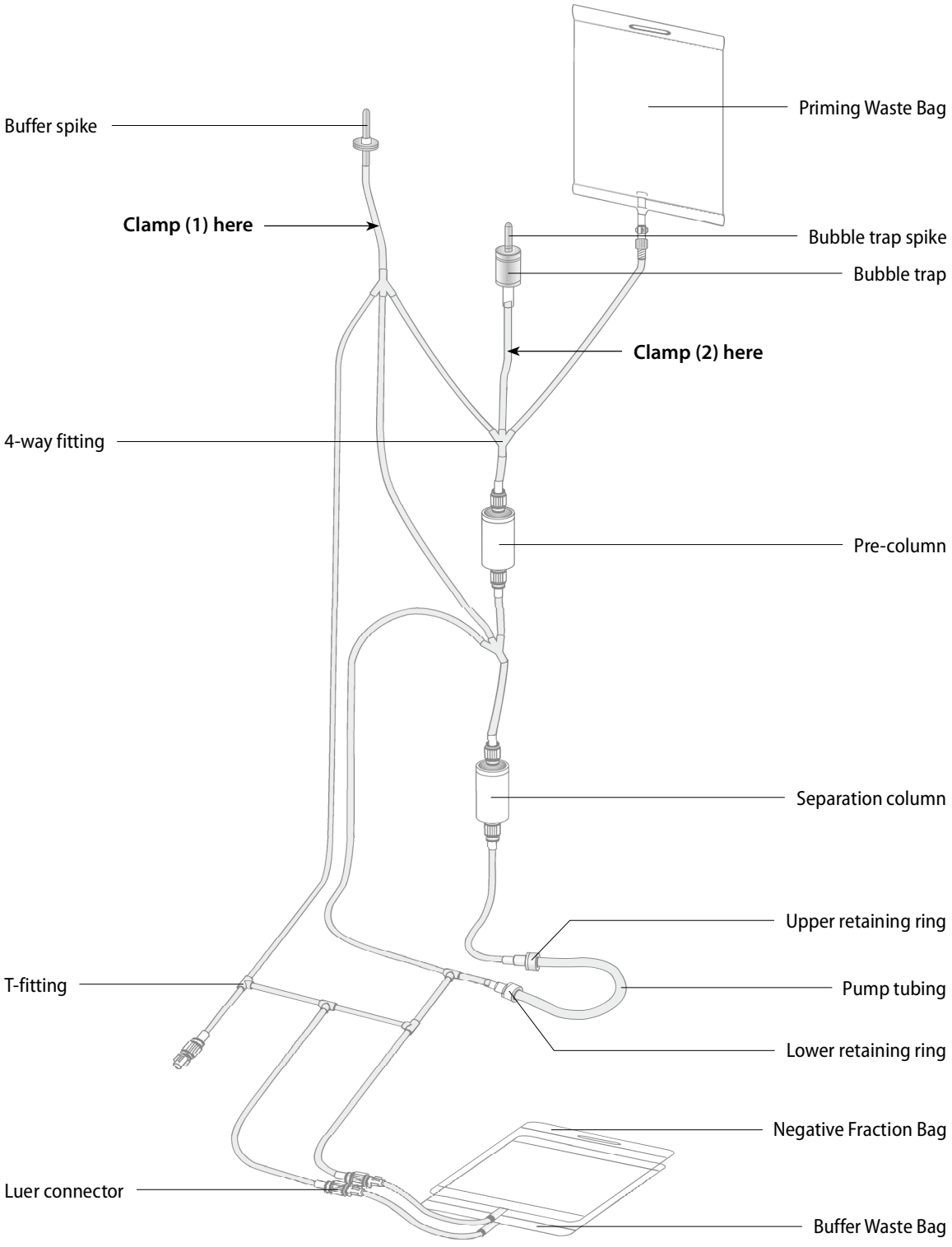


Fig. 4.3-10: General construction of a CliniMACS Tubing Set (e.g. CliniMACS Tubing Set TS, REF 161-01).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

STEP 3
Installation of the CliniMACS® Depletion Tubing Set

Follow the installation instructions given on the screens of the CliniMACS® plus Instrument.

(1) Install upper part.
(2) Install lower part.

Proceed to the priming procedure.

(3) Perform integrity test.

WARNING:

- **To perform the integrity test, clamp the tubing below the Non-Target Cell Bag, before starting the integrity test.**
- **Remove the clamp after completion of the integrity test.**

(4) Connect the Cell Preparation Bag:

- Firmly insert the spike of the pre-system filter into the tubing set,
- Connect pre-system filter and spike connector,
- Spike Cell Preparation Bag,
- Ensure that the connections are secure and that free liquid flow is possible.

(5) Leveling of bags:

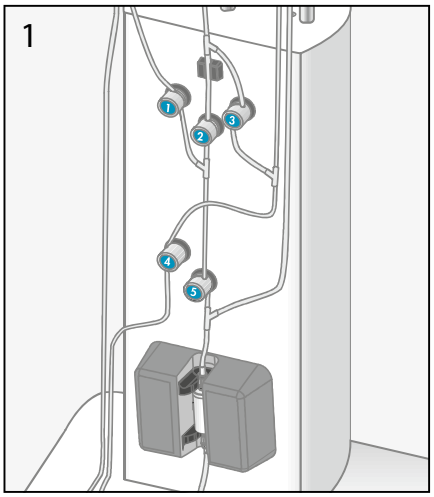
VERY IMPORTANT

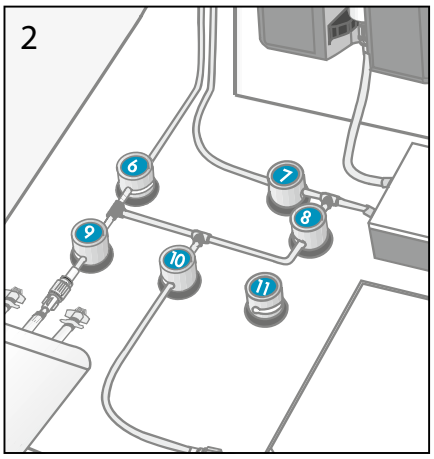
Ensure that the buffer bag, the Cell Preparation Bag, the Non-Target Cell Bag and the Reapplication Bag are leveled correctly as displayed and that the bags and tubings are neither stretched nor bent.

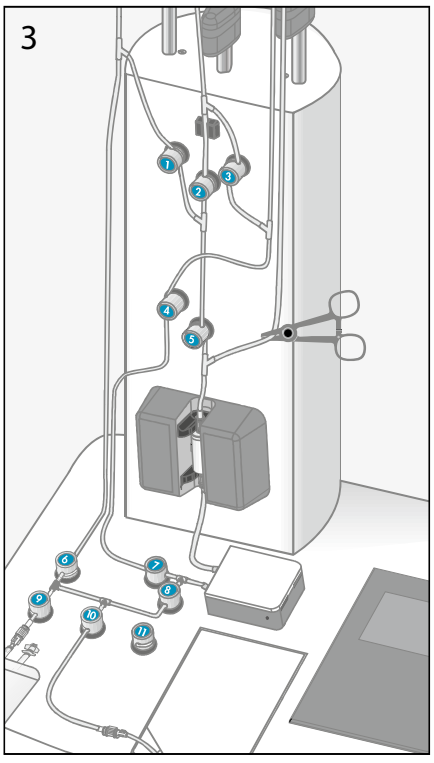
Highest position: Buffer bag

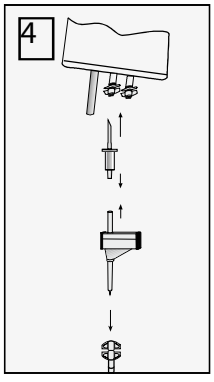
Middle Position: Reapplication Bag and Non-Target Cell Bag

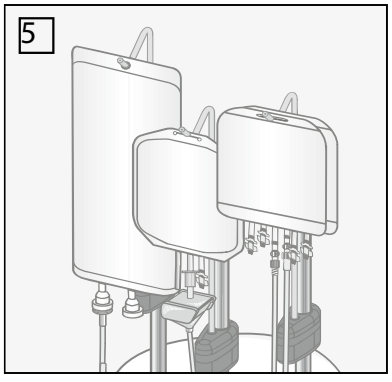
Lowest position: Cell Preparation Bag





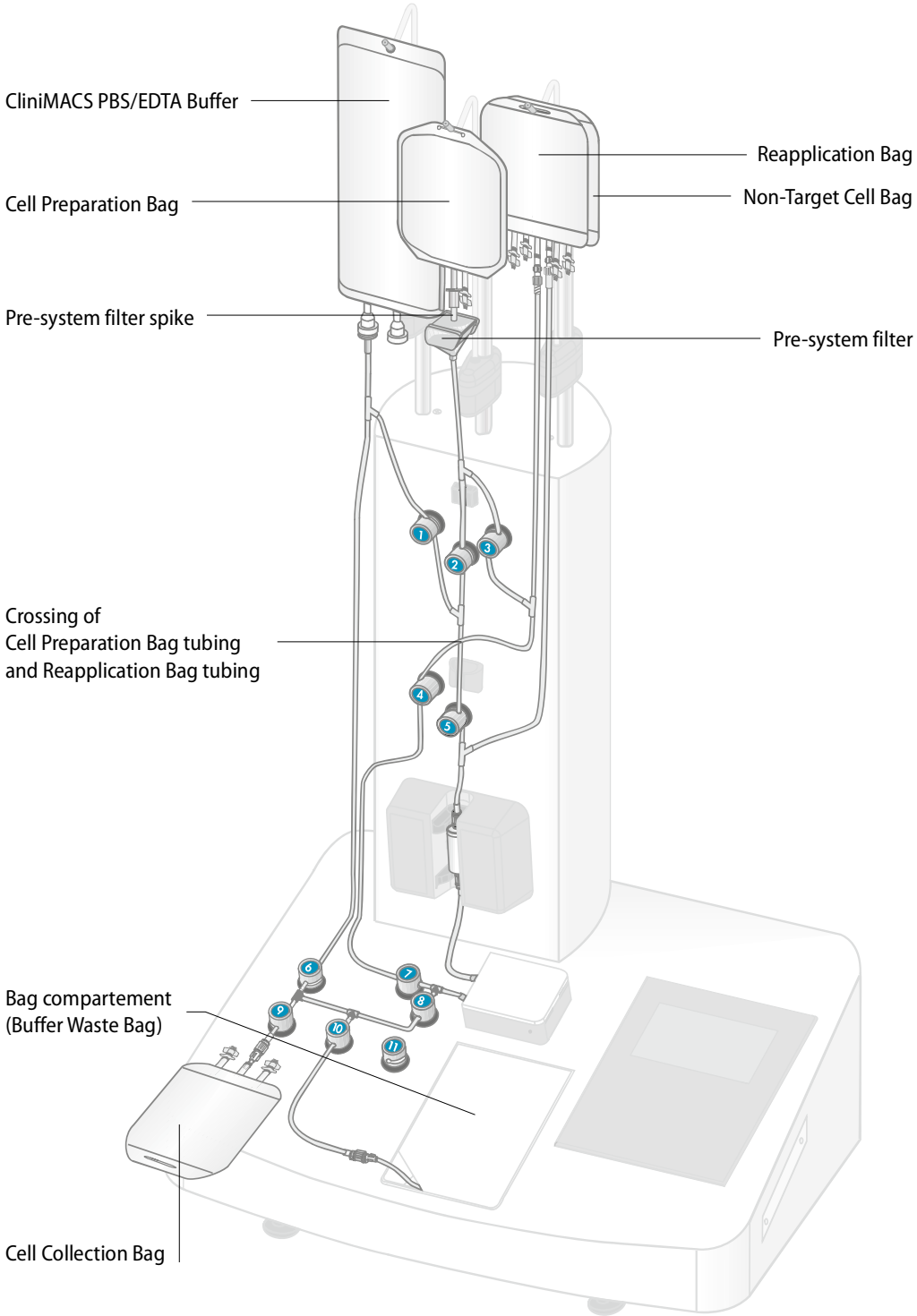






CliniMACS® Depletion Tubing Set

Installed on the CliniMACS® plus Instrument



37000
ch15/15
(Issued:
2015-08)

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

STEP 3:

CliniMACS® Depletion Tubing Set (REF 261-01)

Preparation for tubing set installation

The window will display screen no. 4.3-1 as shown.

Instructions are provided on the right side of the screen and a diagram corresponding to the instruction is displayed on the left. The blinking features on the screen indicate the areas requiring attention.

The CliniMACS® Depletion Tubing Set is provided in a sealed, sterilized package. Each tubing set contains pre-assembled tubings, a column and bags for one cell separation (see fig. 4.3-3). When the packaging is intact, a sterile fluid path is provided.

1. Record the lot number and use-by date of the tubing set. Unpack the sterile tubing set under sterile conditions (e.g. laminar flow hood). Remove the pinch clamp used for packaging before usage of the tubing set. The clamp is labeled with "Remove before assembly to CliniMACS® plus Instrument".

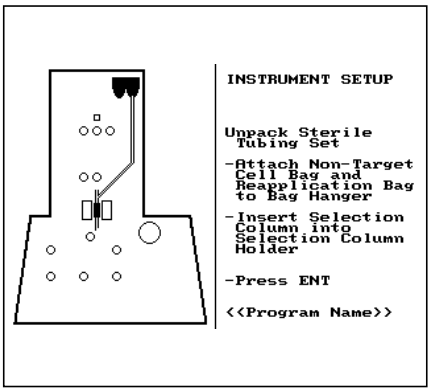
2. Check luer lock connections to bags. Luer locks must be closed tightly.

Cell Collection Bag

The CliniMACS Depletion Tubing Set is provided with an attached Cell Collection Bag.

The weight of the empty Cell Collection Bag attached is 32 g. In case of bag replacement, determine and record the weight of the new empty bag. Attach the sterile Cell Collection Bag to the luer connector on the tubing set before loading the tubing set onto the CliniMACS plus Instrument.

If more than one Cell Collection Bag is necessary for a separation, connect the bags using a plasma transfer set. Confirm that all connections are closed tightly.



Screen no. 4.3-1

Note

- The CliniMACS plus Instrument shows the chosen program name, e.g. DEPLETION 3.1, in the bottom line.

- At any step during the tubing set installation the Undo Key (see chapter 1, fig. 1.16) can be pushed to return to the previous step.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

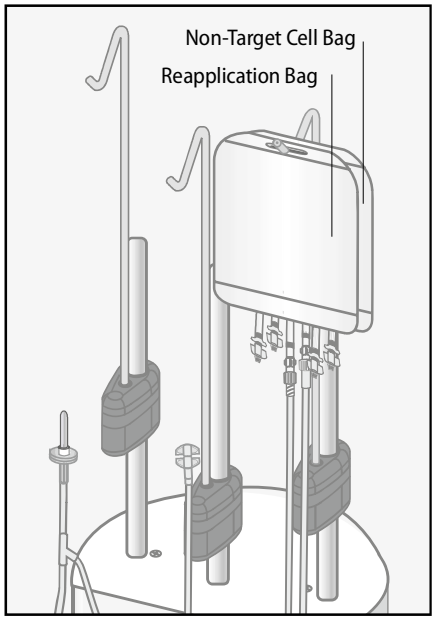


Fig. 4.3-1: Attach Non-Target Cell Bag and Reapplication Bag to bag hanger.

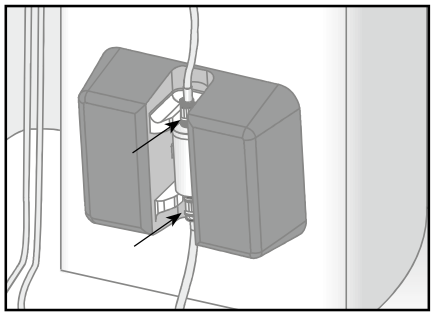


Fig. 4.3-2: Separation column in separation column holder.

Note

- The bag hangers are designed for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.

Attach Non-Target Cell Bag, Reapplication Bag, and insert separation column

1. Attach the Non-Target Cell Bag and the Reapplication Bag to the right hand bag hanger on the instrument as shown (see fig. 4.3-1).

2. Adjust the height of the bag hangers. Raise or lower the hangers to accommodate the height to the size of the Non-Target Cell Bag and Reapplication Bag. Ensure that they are positioned high enough to prevent severe bending of the tubing that could restrict the flow of liquid and that it is low enough to avoid the tubing or connections being stretched.

3. Insert the separation column into the separation column holder as shown (see fig 4.3-2).

Note

To avoid possible pinch injury, insert the separation column as follows: Hold the top and bottom of the column between thumb and index finger, then carefully insert the separation column into the separation column holder.

To proceed, press ENT

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

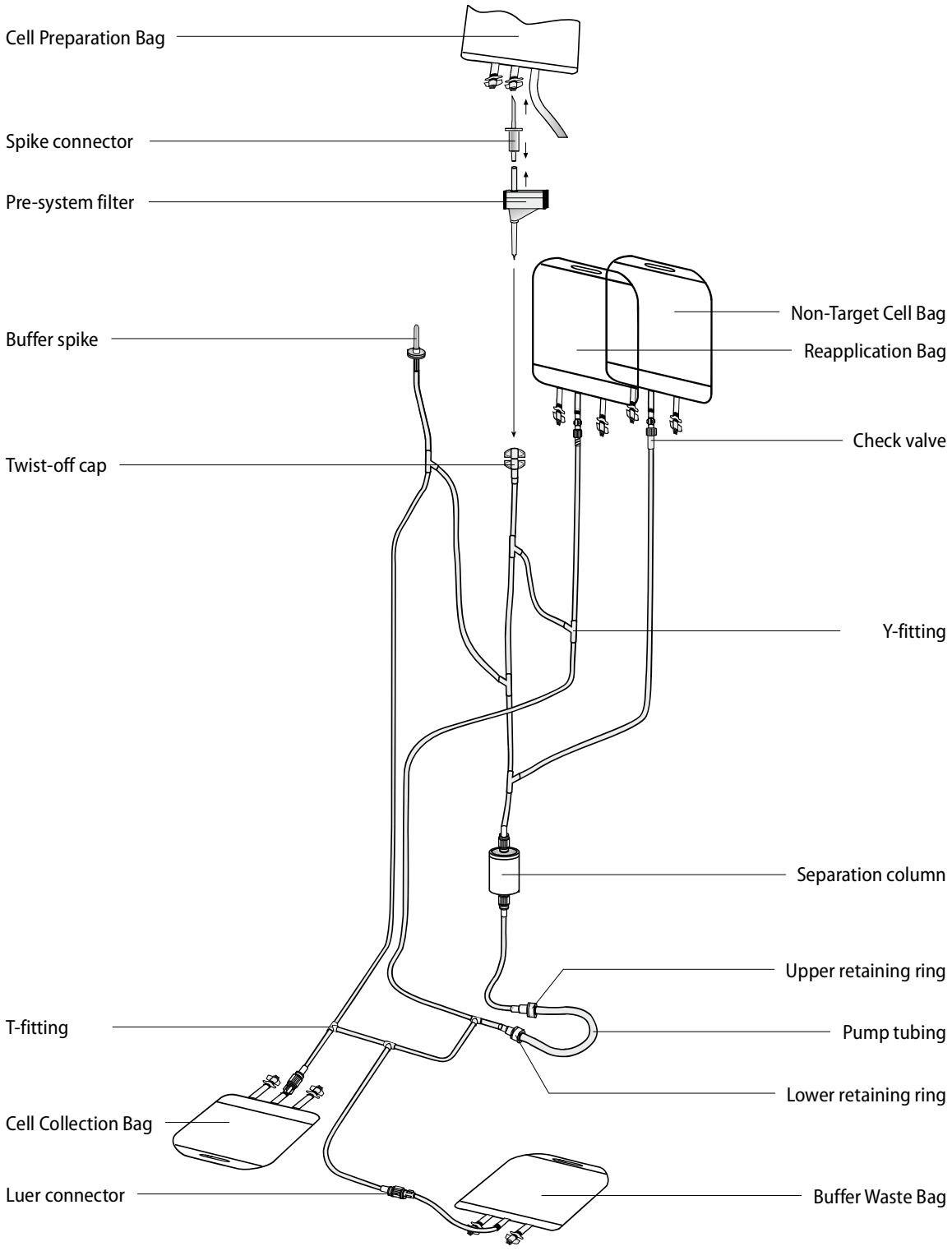
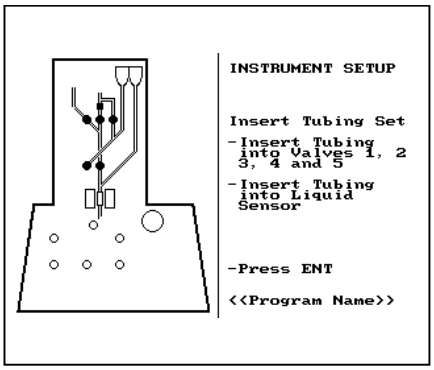


Fig. 4.3-3: General construction of a CliniMACS Depletion Tubing Set (REF 261-01)

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.3-2

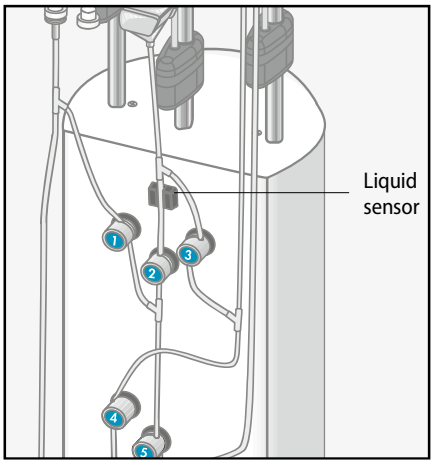


Fig. 4.3-4: Tubing in valves

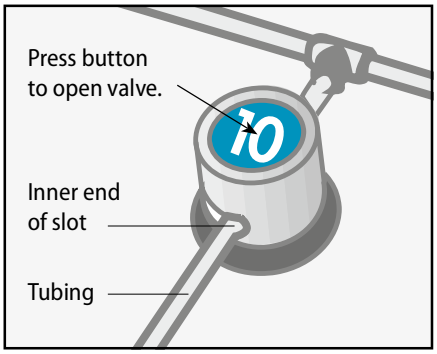


Fig. 4.3-5: Correctly inserted tubing.

Load valves nos. 1, 2, 3, 4, and 5

The window will display screen no. 4.3-2 as shown.

The valves shown on the screen are opened automatically.

1. Load the tubing into valves nos. 1, 2, 3, 4, and 5.

2. Mount the tubing between valve no. 2 and the twist-off cap into the liquid sensor (fig. 4.3-4). Ascertain that the tubing is placed correctly into the sensor fitting.

Note

To ensure proper operation, both the liquid sensor and the tubing being inserted MUST BE DRY. Carefully inspect both. If any liquid is present, dry the area with a soft, lint-free cloth.

To proceed, press ENT

Note

- As each step is performed, check all tubing and attachments for any kinks or severe bending that could restrict the flow of liquid through the tubing. Check all valves to ensure the tubing fits snugly.

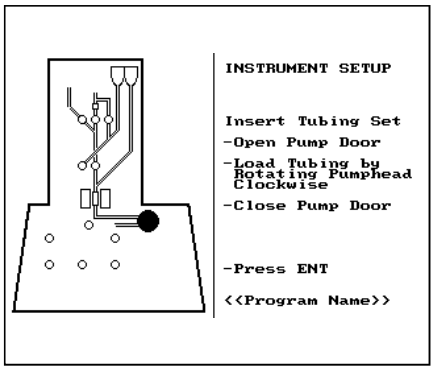
- Only insert the tubing set into open valves (when button is pushed inwards). The tubing will not fit correctly if inserted into a closed valve.

- If the tubing has to be adjusted after a valve has been closed, do not pull the tubing without pressing the valve button to open the valve (see fig. 4.3-5).

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Load pump tubing

The window will display screen no. 4.3-3 as shown.



Screen no. 4.3-3

1. Open the pump door by lifting up at the left hand edge.
2. Insert the upper retaining ring of the pump tubing into the retaining ring groove in the pump housing (see fig. 4.3-6).
3. Rotate the pump roller clockwise (see fig. 4.3-6) until the tubing is threaded between both sets of the tubing guide pins and the tubing fits snugly around the pump roller. Ensure the tubing is not pinched at the end of the guide pins (If adjustment of the tubing inside the pump is necessary, the tubing can be unloaded by lifting the lower end and turning the pump roller anti-clockwise.).
4. Insert the lower retaining ring of the pump tubing into the retaining ring groove in the pump housing (see fig. 4.3-6).
5. Repeat clockwise rotation of the pump roller to confirm that the pump roller moves freely.
6. Close the pump door.

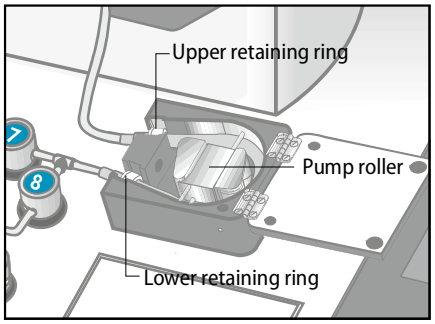


Fig. 4.3-6: Loading of pump tubing.

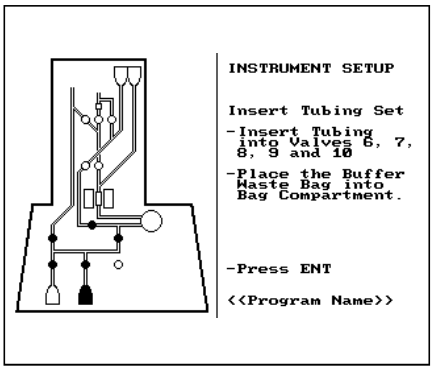
Note

During the cell separation the pump will immediately stop the run whenever the pump housing is opened. If left open for more than 600 seconds the instrument will abort the run in progress.

To proceed, press ENT

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Load valves nos. 6, 7, 8, 9, and 10



Screen no. 4.3-4

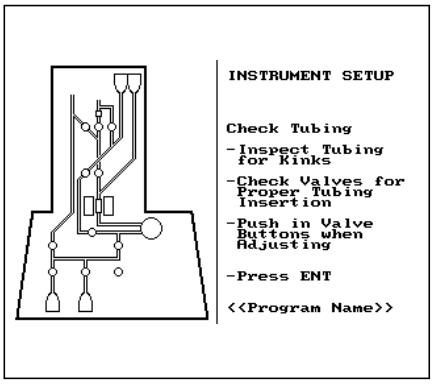
The window will display screen no. 4.3-4 as shown.

1. Load the tubing into valve nos. 6, 7, 8, 9, and 10. Ascertain that the tubing is placed securely in the valve opening.

2. Place the Buffer Waste Bag in the bag compartment. Confirm the tubing is not compressed under the bag compartment lid.

To proceed, press ENT

Recheck all tubing and attachments



Screen no. 4.3-5

The window will display screen no. 4.3-5 as shown.

1. Beginning with valve no. 1, verify that the tubing fits properly and is positioned in each valve correctly.

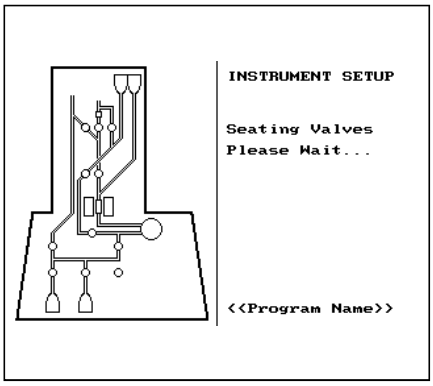
2. Reinspect the tubing in each valve. Confirm that the tubing enters and leaves each valve through the enlargement at the inner end of the slot and is positioned in the center of the jaws of the valve (see fig. 4.3-5). Check that the tubing is not kinked or twisted and does not show any tendency to move away from the center of the pinch valve.

Note

If the tubing has to be adjusted after a valve has been closed, do not pull the tubing without pressing the valve button to open the valve.

To proceed, press ENT

Seating of valves



Screen no. 4.3-6

The window will display screen no. 4.3-6 as shown.

In order to ensure the proper fitting of tubing in the valves, the instrument will operate all of the valves in sequence, twice. Watch and listen to confirm all valves are working properly. If any valve does not operate correctly, refer to the trouble-shooting section. This step may be repeated by using the Undo Key followed by the Enter Key (see chapter 1, fig. 1.16).

The magnet drive is also be tested during this check sequence.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Attach CliniMACS® PBS/EDTA Buffer

The window will display screen no. 4.3-7 as shown.

The prescribed buffer for CliniMACS® Separations is CliniMACS PBS/EDTA Buffer supplemented with HSA to a final concentration of 0.5% (w/v).

1. Using aseptic technique, remove the cap from the buffer spike (see fig. 4.3-3) on the tubing set and connect it to the buffer bag. Ensure that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to ascertain that the spike has penetrated the bag.

2. Attach the buffer bag to the buffer bag hook on the bag hanger (see fig. 4.3-7).

3. Adjust the height of the buffer bag hanger. Raise or lower the hanger to accommodate the size of the buffer bag. Ensure that it is positioned high enough to prevent severe bending of the tubing that could restrict the flow of fluid and that it is low enough to avoid the tubing or connections being stretched (see fig. 4.3-7).

To proceed, press ENT

Important

Due to the gravimetric rinsing steps, it is important that the buffer bag is positioned higher than the Reapplication Bag and the Non-Target Cell Bag (see fig. 4.3-8).

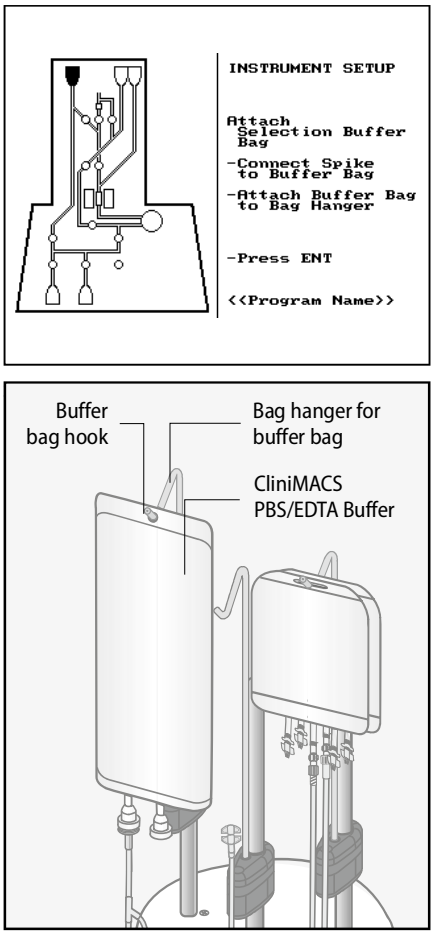
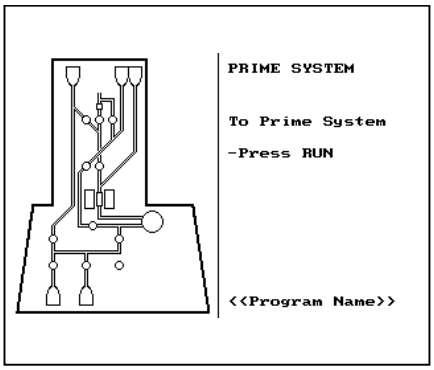


Fig. 4.3-7: Attach CliniMACS PBS/EDTA Buffer to bag hanger.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Start priming



Screen no. 4.3-8

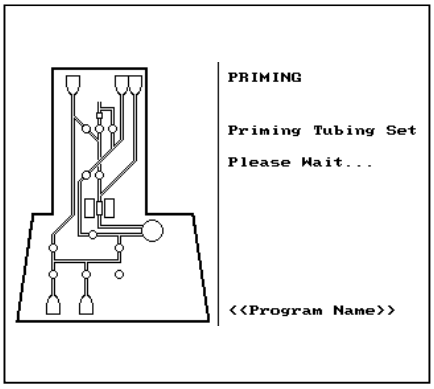
The window will display screen no. 4.3-8 as shown.

To start priming, press RUN

The window will display screen no. 4.3-9 as shown.

During the priming phase the tubing set is filled with CliniMACS PBS/EDTA Buffer. The buffer is circulated through the tubing set including the separation column. The priming waste is collected in the Buffer Waste Bag, Reapplication Bag and the Non-Target Cell Bag (see fig. 4.3-3). The priming cycles will continue, repeating a series of steps. The priming phase takes approximately 2.5 minutes. Priming status is then updated on the display.

Check during the priming



Screen no. 4.3-9

During the priming phase, check all tubing, fittings, valves and the separation column for the appearance of any leaks or the presence of any folds that may block fluid flow.

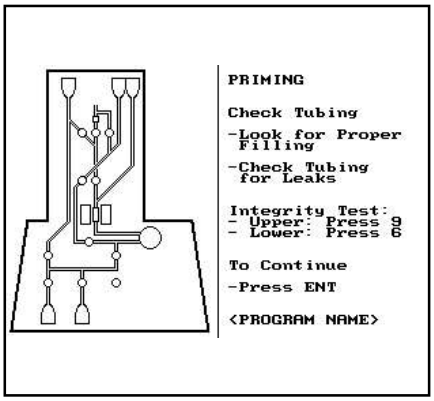
If leaks or malfunctions are observed, stop run by pressing 'STOP'. The operator has 600 seconds to resolve the problem. Restart the process by pressing 'RUN'.

After 600 seconds, the separation will be automatically aborted. If the operator is unable resolve the problem or if the tubing set is defective, remove the tubing set and replace it with a new one. Maintain the tubing set and contact Miltenyi Biotec's Customer Service department for instructions regarding the return of the defective tubing set.

Note

Once priming has started, it is not possible to return to the instrument set-up procedure.

Final check of all tubing and attachments



Screen no. 4.3-10

The window will display screen no. 4.3-10 as shown.

Before beginning the run, check the following:

- fluid in all parts of tubing set except for tubing above valves 2 and 3,
- no excess air in tubing set,
- fluid in Reapplication Bag, Buffer Waste Bag and Non-Target Cell Bag,
- **no** fluid in the Cell Collection Bag.

DO NOT press 'ENT' yet.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Integrity test

For additional safety, an integrity test must be performed to test the tubing set for leaks. The test sequence consists of two automated sequences, which allow both the upper and the lower parts of the tubing set to be over pressurized and tested separately

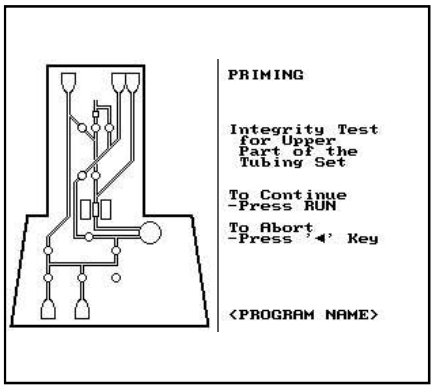
Integrity test for the upper part of the tubing set

1. When the operator performs "Final check of all tubing and attachments" the window displays screen no. 4.3-10 as shown.

2. After performing the "Final check of all tubing and attachments", DO NOT press 'ENT'.

3. To enter the integrity test for the upper part, press [9]

4. The window will display screen no. 4.3-11 as shown.



Screen no. 4.3-11

Important

Clamp the tubing underneath the check valve of the Non-Target Cell Bag.

5. To start the test sequence, press [RUN]

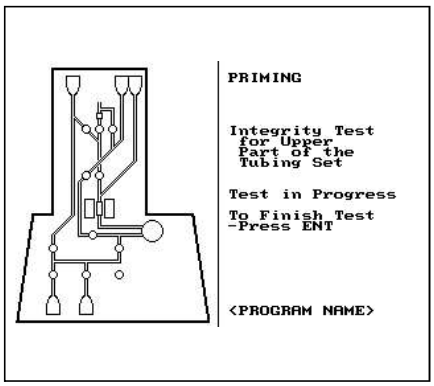
   To return to screen no. 4.3-10, press [T]

6. Once the "RUN" button has been pressed, the instrument starts the automated test sequence for the upper part of the tubing set.

   The window will display screen no. 4.3-12 as shown.

   Overpressure will be created and held for 2 minutes. During this time the operator should watch the connections above and under the separation column and the upper pump tube connection.

   At each point the test sequence can be finished by pressing 'ENT'.



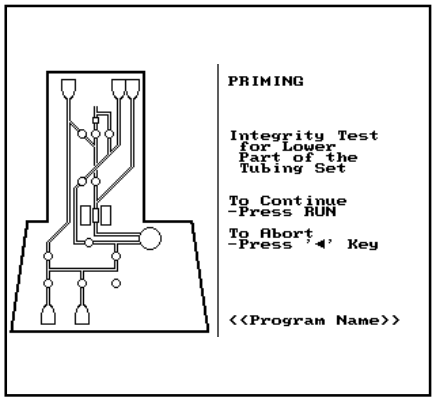
Screen no. 4.3-12

7. After 2 minutes the pressure is automatically released and the window displays screen no. 4.3-10.

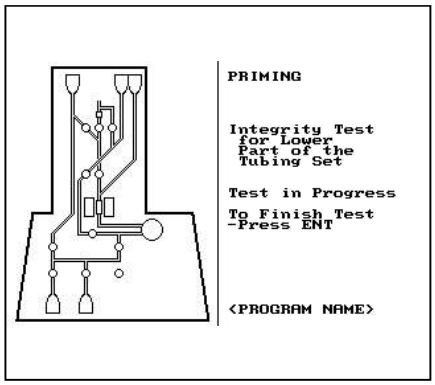
   Using a tissue, the operator should check to determine if any leaks have occured during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one. Contact Miltenyi Biotec's Customer Service department for instructions regarding the return of the defective tubing set.

8. If no leakages are observed, continue with the integrity test of the lower part of the tubing set.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.3-13



Screen no. 4.3-14

Important

- Remove the clamp when the integrity test is finished.

Integrity test for the lower part of the tubing set

1. The window will display screen no. 4.3-10 as shown.
2. To enter the integrity test, press [6]
   DO NOT press "ENT".
3. The window will display screen no. 4.3-13 as shown.
4. To start the test sequence, press [RUN]

   To go back to screen no. 4.3-10, press [T]
5. Once the RUN button has been pressed, the instrument starts the automated test sequence for the lower part of the tubing set.

   The window will display screen no. 4.3-14 as shown.

   Overpressure will be created and held for 30 seconds. During this time the operator should watch the lower pump tube connection and the T-fittings between valves nos. 6, 8, 9, and 10.

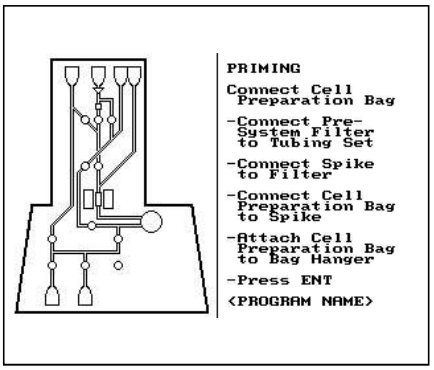
   At each point the test sequencecan be finished by pressing 'ENT'.
6. After 30 seconds the pressure is automatically released and the window displays screen no. 4.3-10.

   Using a tissue the operator should now check if any leakages have occured during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one. Contact Miltenyi Biotec's Customer Service department for instructions regarding the return of the defective tubing set.
7. **Open the pathway to the Non-Target Cell Bag by removing the clamp underneath the check valve.**
8. If no leakages are observed, the operator can now continue with the next step by pressing [ENT]

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Connect Cell Preparation Bag

The window will display screen no. 4.3-15 as shown.



Screen no. 4.3-15

After the priming phase has been completed and no leaks or malfunctions are observed, the Cell Preparation Bag can be attached (see fig. 4.3-8). Use aseptic techniques for all steps.

Connect the Cell Preparation Bag containing the magnetically labeled and washed cells with the tubing set:

1. Remove the twist-off cap (see fig. 4.3-3) from the tubing set.

2. Remove the cap from the spike of the pre-system filter (see fig. 4.3-3). Firmly insert the spike of the pre-system filter into the tubing set, ensuring the septum is punctured.

3. Remove the caps from the pre-system filter and the blunt end of the spike connector (see fig. 4.3-3) and connect both parts.

4. Remove the other cap from the spike connector and connect the spike to the Cell Preparation Bag (see fig. 4.3-3) ensuring that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to ascertain that the spike has penetrated the bag.

5. Check the connection between the Cell Preparation Bag, spike connector, the pre-system filter and the tubing set to confirm that the connection is secure.

6. Hang the Cell Preparation Bag on the bag hanger (see fig. 4.3-9).

7. Confirm the bags and tubings attached to the bag hanger are neither streched nor bent.

   Due to the gravimetric rinsing steps performed by the instrument during the automated separation it is VERY IMPORTANT that the bag are leveled correctly (see fig 4.3-8):

   - **Highest position**: buffer bag,
   - **Middle position:** Reapplication and Non-Target Cell Bag,
   - **Lowest position:** Cell Preparation Bag.

To proceed, press ENT

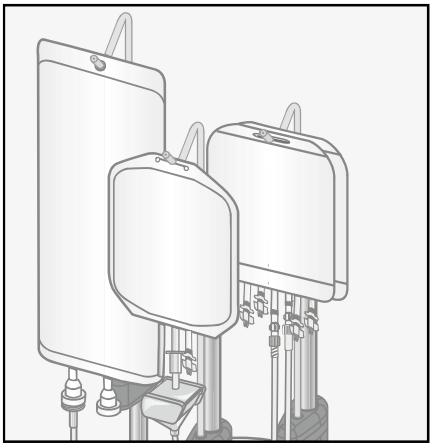
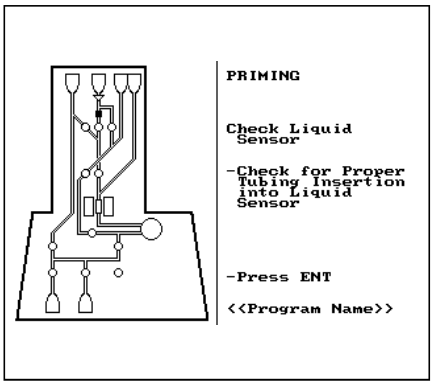


Fig. 4.3-8: Correctly leveled bags.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.3-16

Final check of the liquid sensor

The window will display screen no. 4.3-16 as shown.

1. Check the liquid sensor tubing. Ensure the tubing has been properly inserted, that it is free of any external liquid and has not been dislodged during the loading procedure.

2. Confirm that the unrestricted flow of fluid is possible to each bag.

To proceed, press ENT

Proceed to STEP 4.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

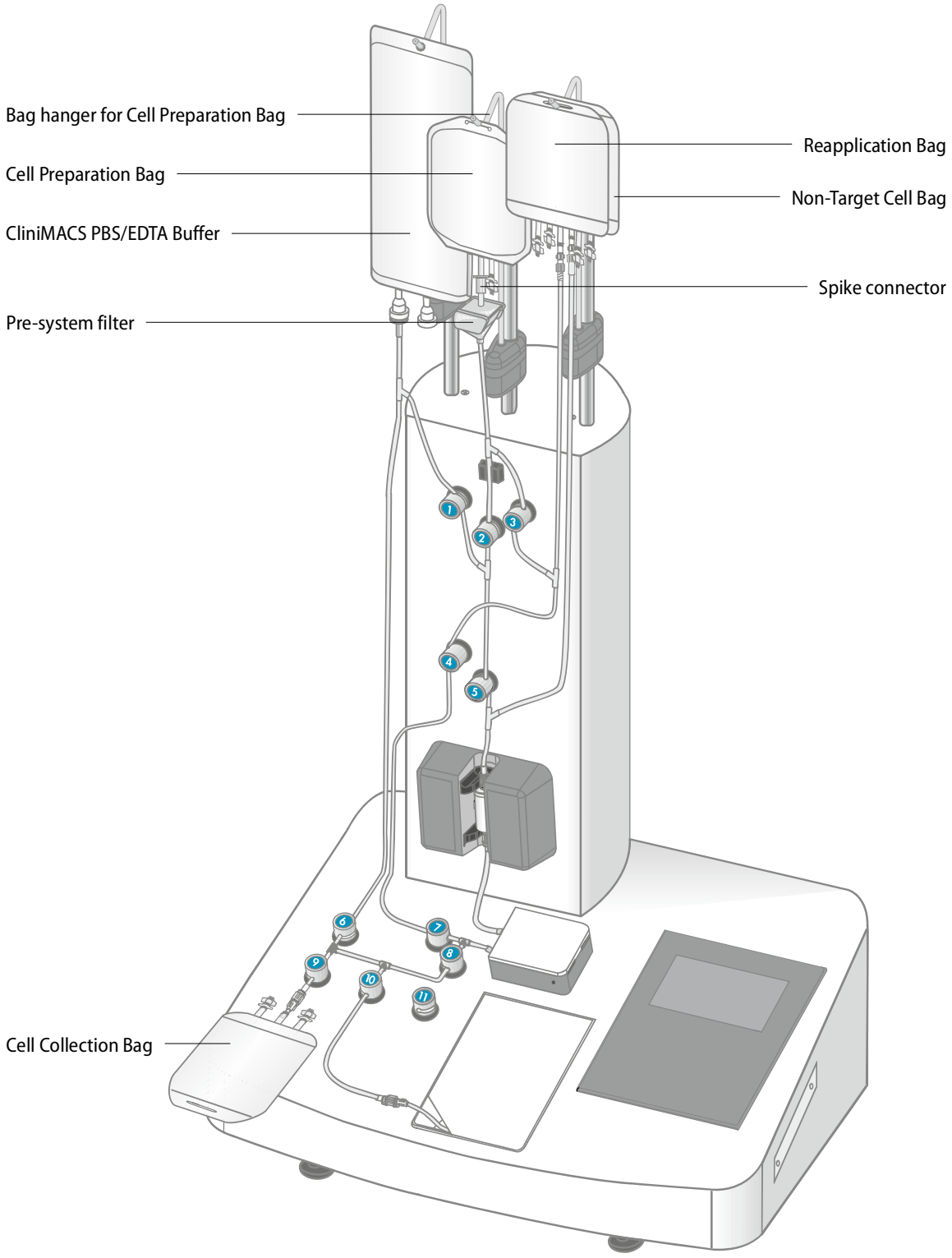


Fig. 4.3-9: CliniMACS plus Instrument with CliniMACS Depletion Tubing Set, CliniMACS PBS/EDTA Buffer, Cell Preparation Bag and Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

STEP 4:

CD34 SELECTION 1/2

Once the final check has been completed, the CliniMACS plus Instrument is ready to begin the separation, the window will display screen no. 4.4-1 as shown.

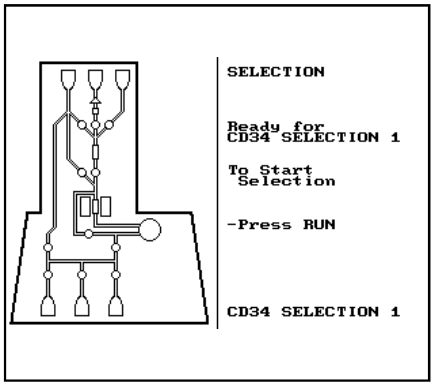
Make a final check of all tubing and attachments.

To proceed, press RUN

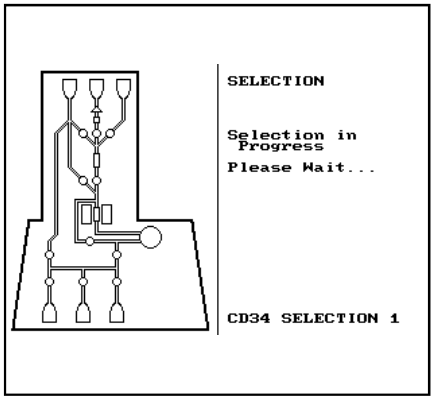
Once 'RUN' has been pressed, the instrument will automatically perform the separation procedure chosen.

At each phase of the operation, a screen similar to screen no. 4.4-2 is displayed to show the status of the separation procedure.

The magnet position indicator is displayed as two black boxes next to the separation column when the magnet is "ON", meaning it has been moved to the front to magnetize the separation column. If the magnet position indicator is transparent (e.g. screen no. 4.4-1), the magnet is "OFF", meaning it has been moved to the rear of the instrument. With the magnet withdrawn the separation column is outside the magnetic field and is not magnetized.



Screen no. 4.4-1



Screen no. 4.4-2

Separation procedure

The general steps of the separation procedure are the same for both CD34 separation programs (CD34 SELECTION 1 and 2).

Loading cells

The separation procedure starts with the filling of the pre-system filter to complete the priming of the system. Buffer is pumped upwards towards the Cell Preparation Bag to fill the pre-system filter. Tap the side of the filter several times to remove any bubbles which might be trapped in the filter. Then the separation of the labeled cells begins. The pump draws the contents of the Cell Preparation Bag into the tubing set. The magnetically labeled cells are retained in the separation column, placed in the magnetic field, while the unlabeled cells (non-target cells) are passed through and collected in the Negative Fraction Bag. When the Cell Preparation Bag is empty (detected automatically by the liquid sensor) the pre-system filter is rinsed twice with buffer.

Note

At each phase of the operation, the status of the separation procedure is shown on the screen.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Column wash I

The pre-column and separation column are washed extensively to remove all unlabeled cells. Wash buffer is collected in the Buffer Waste Bag. When 'Column Wash I' starts, the total remaining time until the end of the separation procedure is shown.

Release of cells I

The magnet is moved to the rear of the instrument ("OFF" position). The retained cells are released at a high speed flow, but the cells remain within an internal tubing cycle.

Reloading of cells I

The magnet is moved into the "ON" position again to magnetize the separation column and the cells are reapplied on the separation column.

Column wash II

Reloading of the cells is followed by a second washing step to remove remaining unlabeled cells. Also, all tubing is rinsed several times.

Release of cells II, reloading of cells II, column wash III

The cells are released and reapplied on the separation column for a second time in order to remove any unlabeled cells that only stick to the column matrix. Afterwards the separation column is washed again.

Release of cells III, reloading of cells III, column wash IV

Additionally, the separation program CD34 SELECTION 2 includes a third release and reapplication step.

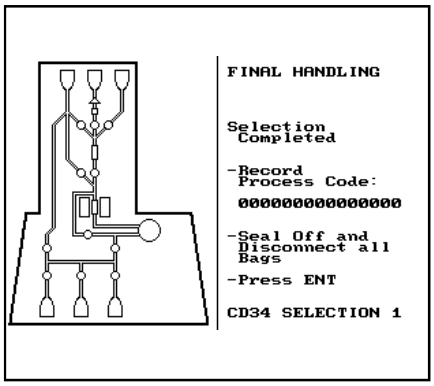
Final elution of the cells

The magnet is moved into the "OFF" position and the magnetically labeled CD34 positive cells are released from the separation column and collected in the attached Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Disconnect bags and record process code

When the run has been completed, the CliniMACS plus Instrument will display screen no. 4.4-3 as shown.



Screen no. 4.4-3

1. Record the process code on the worksheet (C_7).

2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see fig. 4.4-1). Using the heat sealer, make three hermetic seals in the tubing directly below valve no. 9. Carefully sever the middle seal to disconnect the Cell Collection Bag from the tubing set.

3. Weigh the filled Cell Collection Bag. Record the weight on the worksheet (C_4). Determine the weight of the target cell fraction by subtracting the weight of the empty Cell Collection Bag (C_5) from the weight of the Cell Collection Bag containing the target cells (C_4). Record the weight on the worksheet (C_6).

Worksheet equation:
$C_6 = C_4 - C_5$

4. Mix the target cell suspension thoroughly by rotating the bag. Take an aliquot of 0.5 mL and retain for analysis.

5. Using the heat sealer, seal off the tubing above the luer lock of the Negative Fraction Bag (see fig 4.4-1). Make three hermetic seals in the tubing. Sever the center seal to disconnect the Negative Fraction Bag.

6. Disconnect the Buffer Waste Bag in the same way (see fig. 4.4-1).

7. Remove the Negative Fraction Bag and Buffer Waste Bag. Keep all bags until analysis of all cells has been completed.

The target cells can now be processed in accordance with clinical protocols.

To proceed, press ENT

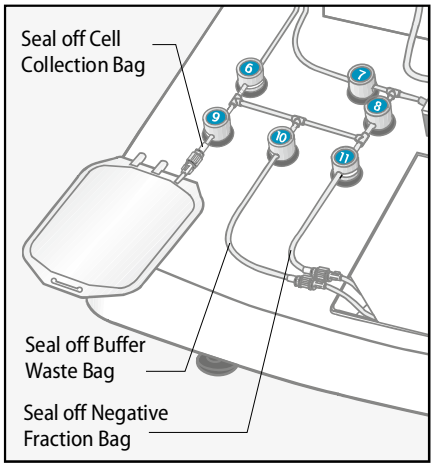
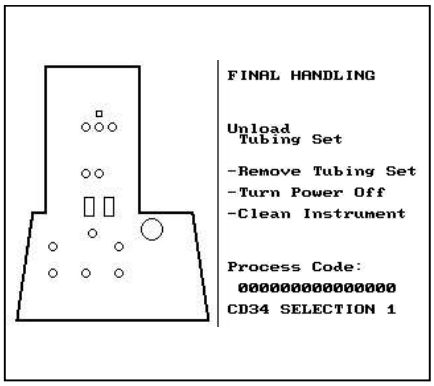


Fig. 4.4-1: Where to disconnect the tubing set after the run has been completed. Seal off above luer lock.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.4-4

Important

Prior to target cell infusion the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans. The user of the CliniMACS System is responsible for the selection of an appropriate medium for infusion.

If the volume of the target cell fraction exceeds 100 mL, it must be reduced by appropriate means, e.g. through centrifugation or buffer exchange to an infusion solution approved in your country.

Unload tubing set and shutdown

The window will display screen no. 4.4-4 as shown.

1. Remove the tubing set:
   Beginning with valve nos. 6, 9, 10 and 11, and working upwards, release the tubing from the liquid sensor and from the valves, by pressing on the valves. Release the columns from the column holders. Dispose the tubing set as a biohazard, according to standard hospital procedures.

2. Switch off the CliniMACS[plus] Instrument.

3. Clean the instrument according to cleaning instructions, see chapter 1.3.6. Follow the standard procedures for the treatment of infectious material.

Analysis of cells

The target cells must be examined regarding quality and quantity in view of their intended use.

This must include the following parameters:

- Total number of leukocytes,
- Viability and total number of CD34 positive cells,
- Purity and recovery of CD34 positive cells.

It is also recommended to determine the total number of leukocytes and the viability of the non-target cell fraction.

The list is an example and other tests should be included based on the intended use and clinical protocols.

Record the analysis data on the worksheet.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

STEP 4:

CD133 SELECTION 1/2

Once the final check has been completed, the CliniMACS plus Instrument is ready to begin the separation, the window will display screen no. 4.4-1 as shown.

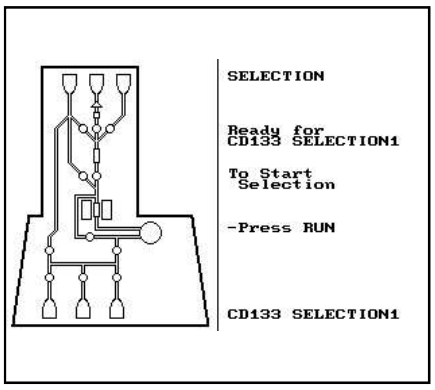
Make a final check of all tubing and attachments.

To proceed, press RUN

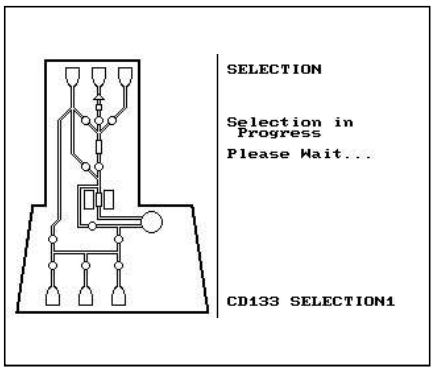
Once 'RUN' has been pressed, the instrument will automatically perform the separation procedure chosen.

At each phase of the operation, a screen similar to screen no. 4.4-2 is displayed to show the status of the separation procedure.

The magnet position indicator is displayed as two black boxes next to the separation column when the magnet is "ON", meaning it has been moved to the front to magnetize the separation column. If the magnet position indicator is transparent (e.g. screen no. 4.4-1), the magnet is "OFF", meaning it has been moved to the rear of the instrument. With the magnet withdrawn the separation column is outside the magnetic field and is not magnetized.



Screen no. 4.4-1



Screen no. 4.4-2

Separation procedure

The general steps of the separation procedure are the same for both CD133 separation programs (CD133 SELECTION 1 and 2).

Loading cells

The separation procedure starts with the filling of the pre-system filter to complete the priming of the system. Buffer is pumped upwards towards the Cell Preparation Bag to fill the pre-system filter. Tap the side of the filter several times to remove any bubbles which might be trapped in the filter. Then the separation of the labeled cells begins. The pump draws the contents of the Cell Preparation Bag into the tubing set. The magnetically labeled cells are retained in the separation column, placed in the magnetic field, while the unlabeled cells (non-target cells) are passed through and collected in the Negative Fraction Bag. When the Cell Preparation Bag is empty (detected automatically by the liquid sensor) the pre-system filter is rinsed twice with buffer.

Note

At each phase of the operation, the status of the separation procedure is shown on the screen.

Column wash I

The pre-column and separation column are washed extensively to remove all unlabeled cells. Wash buffer is collected in the Buffer Waste Bag. When 'Column Wash I' starts, the total remaining time until the end of the separation procedure is shown.

Release of cells I

The magnet is moved to the rear of the instrument ("OFF" position). The retained cells are released at a high speed flow, but the cells remain within an internal tubing cycle.

Reloading of cells I

The magnet is moved into the "ON" position again to magnetize the separation column and the cells are reapplied on the separation column.

Column wash II

Reloading of the cells is followed by a second washing step to remove remaining unlabeled cells. Also, all tubing is rinsed several times.

Release of cells II, reloading of cells II, column wash III

The cells are released and reapplied on the separation column for a second time in order to remove any unlabeled cells that only stick in the column matrix. Afterwards the separation column is washed again.

Release of cells III, reloading of cells III, column wash IV

Additionally, the separation program CD133 SELECTION 2 includes a third release and reapplication step.

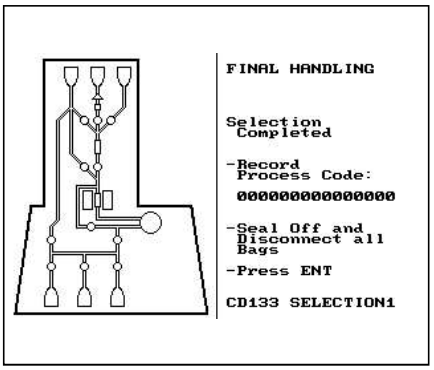
Final elution of the cells

The magnet is moved into the "OFF" position and the magnetically labeled CD133 positive cells are released from the separation column and collected in the attached Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Disconnect bags and record process code

When the run has been completed, the CliniMACS plus Instrument will display screen no. 4.4-3 as shown.



Screen no. 4.4-3

1. Record the process code on the worksheet (C_7).

2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see fig. 4.4-1). Using the heat sealer, make three hermetic seals in the tubing directly below valve no. 9. Carefully sever the middle seal to disconnect the Cell Collection Bag from the tubing set.

3. Weigh the filled Cell Collection Bag. Record the weight on the worksheet (C_4). Determine the weight of the target cell fraction by subtracting the weight of the empty Cell Collection Bag (C_5) from the weight of the Cell Collection Bag containing the target cells (C_4). Record the weight on the worksheet (C_6).

Worksheet equation:
$C_6 = C_4 - C_5$

4. Mix the target cell suspension thoroughly by rotating the bag. Take an aliquot of 0.5 mL and retain for analysis.

5. Using the heat sealer, seal off the tubing above the luer lock of the Negative Fraction Bag (see fig 4.4-1). Make three hermetic seals in the tubing. Sever the center seal to disconnect the Negative Fraction Bag.

6. Disconnect the Buffer Waste Bag in the same way (see fig. 4.4-1).

7. Remove the Negative Fraction Bag and Buffer Waste Bag. Keep all bags until analysis of all cells has been completed.

The target cells can now be processed in accordance with clinical protocols.

To proceed, press ENT

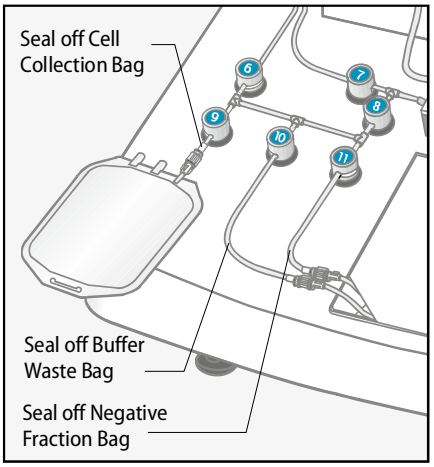
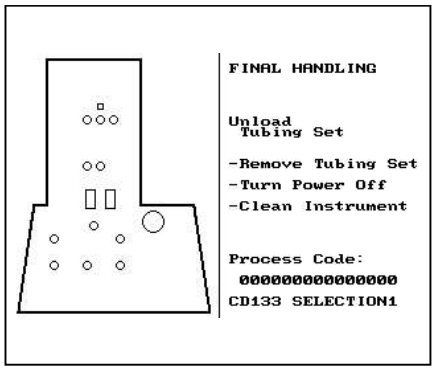


Fig. 4.4-1: Where to disconnect the tubing set after the run has been completed. Seal off above luer lock.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.4-4

Unload tubing set and shutdown

The window will display screen no. 4.4-4 as shown.

1. Remove the tubing set:
   Beginning with valve nos. 6, 9, 10 and 11, and working upwards, release the tubing from the liquid sensor and from the valves, by pressing on the valves. Release the columns from the column holders. Dispose the tubing set as a biohazard, according to standard hospital procedures.

2. Switch off the CliniMACS ^{plus} Instrument.

3. Clean the instrument according to cleaning instructions, see chapter 1.3.6. Follow the standard procedures for the treatment of infectious material.

Important

Prior to target cell infusion the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans. The user of the CliniMACS System is responsible for the selection of an appropriate medium for infusion.

If the volume of the target cell fraction exceeds 100 mL, it must be reduced by appropriate means, e.g. through centrifugation or buffer exchange to an infusion solution approved in your country.

Analysis of cells

The target cells must be examined regarding quality and quantity in view of their intended use. This must include the following parameters:

- Total number of leukocytes,
- Viability and total number of CD133 positive cells,
- Purity and recovery of CD133 positive cells.

It is also recommended to determine the total number of leukocytes and the viability of the non-target cell fraction.

This list is an example and other tests should be included based on the intended use and clinical protocols.

Record the analysis data on the worksheet.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

STEP 4:

ENRICHMENT 1.1

Once the final check has been completed, the CliniMACS plus Instrument is ready to begin the separation, the window will display screen no. 4.4-1 as shown.

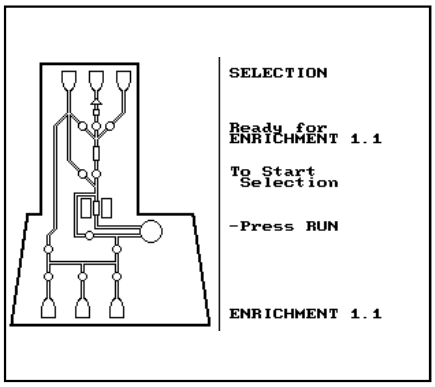
Make a final check of all tubing and attachments.

To proceed, press RUN

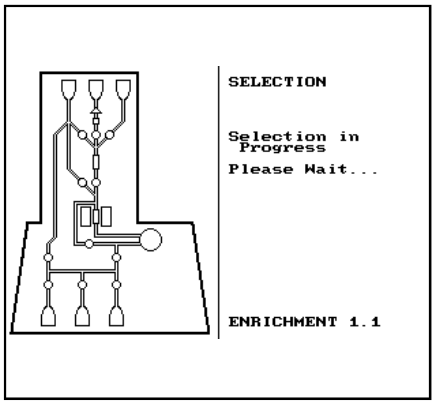
Once 'RUN' has been pressed, the instrument will automatically perform the separation procedure chosen.

At each phase of the operation, a screen similar to screen no. 4.4-2 is displayed to show the status of the separation procedure.

The magnet position indicator is displayed as two black boxes next to the separation column when the magnet is "ON", meaning it has been moved to the front to magnetize the separation column. If the magnet position indicator is transparent (e.g. screen no. 4.4-1), the magnet is "OFF", meaning it has been moved to the rear of the instrument. With the magnet withdrawn the separation column is outside the magnetic field and is not magnetized.



Screen no. 4.4-1



Screen no. 4.4-2

Separation procedure

Loading cells

The separation procedure starts with the filling of the pre-system filter to complete the priming of the system. Buffer is pumped upwards towards the Cell Preparation Bag to fill the pre-system filter. Tap the side of the filter several times to remove any bubbles which might be trapped in the filter. Then the separation of the labeled cells begins. The pump draws the contents of the Cell Preparation Bag into the tubing set. The magnetically labeled cells are retained in the separation column, placed in the magnetic field, while the unlabeled cells (non-target cells) are passed through and collected in the Negative Fraction Bag.

Column wash I

The pre-column and separation column are washed extensively to remove all unlabeled cells. Wash buffer is collected in the Buffer Waste Bag. When 'Column Wash I' starts, the total remaining time until the end of the separation procedure is shown.

Note

At each phase of the operation, the status of the separation procedure is shown on the screen.

Note

- If the number of magnetically labeled cells (calculated by the CliniMACS Software) exceeds the binding capacity of the separation column, the separation program automatically loads and separates the cell sample in smaller portions ("staged loading").

- The loading is stopped when the capacity of the separation column is reached and the separation program then proceeds to the next step of the separation. After the last step (Final elution of the cells) finishes, the next portion of the sample is loaded onto the column. This sequence continues until the entire volume of magnetically labeled cells is loaded onto the separation column.

Release of cells

The magnet is moved to the rear of the instrument ("OFF" position). The retained cells are released at a high speed flow, but the cells remain within an internal tubing cycle.

Reloading of cells

The magnet is moved into the "ON" position again to magnetize the separation column and the cells are reapplied on the separation column.

Column wash II

Reloading of the cells is followed by a second washing step to remove remaining unlabeled cells. Also, all tubing is rinsed several times.

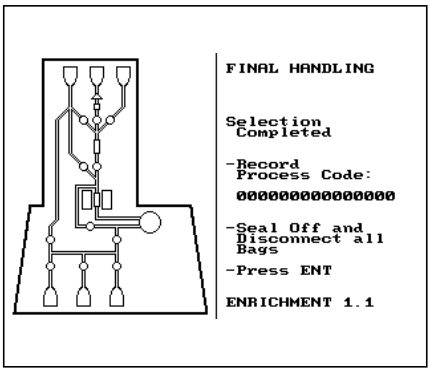
Final elution of the cells

The magnet is moved into the "OFF" position and the magnetically labeled cells (target cells) are released from the separation column and collected in the attached Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Disconnect bags and record process code

When the run has been completed, the window will display screen no. 4.4-3 as shown.



Screen no. 4.4-3

1. Record the process code.

2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see fig. 4.4-1). Using the heat sealer, make three hermetic seals in the tubing directly below valve no. 9. Carefully sever the middle seal to disconnect the Cell Collection Bag from the tubing set.

3. Weigh the filled Cell Collection Bag. Record the weight. Determine the weight of the target cell fraction by subtracting the weight of the empty Cell Collection Bag from the weight of the Cell Collection Bag containing the target cells. Record the weight.

4. Mix the target cell suspension thoroughly by rotating the bag. Take an aliquot of 0.5 mL and retain for analysis.

5. Using the heat sealer, seal off the tubing above the luer lock of the Negative Fraction Bag (see fig 4.4-1). Make three hermetic seals in the tubing. Sever the center seal to disconnect the Negative Fraction Bag.

6. Disconnect the Buffer Waste Bag in the same way (see fig. 4.4-1).

7. Remove the Negative Fraction Bag and Buffer Waste Bag. Keep all bags until analysis of all cells has been completed.

The target cells can now be processed in accordance with clinical protocols.

To proceed, press ENT

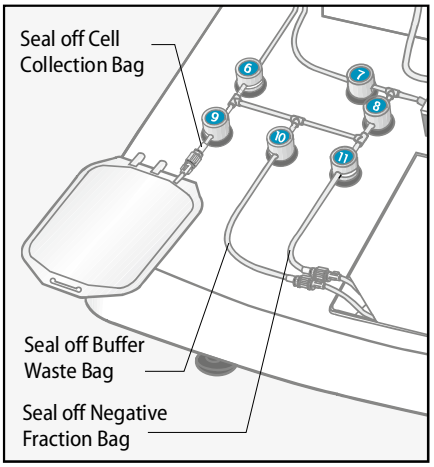
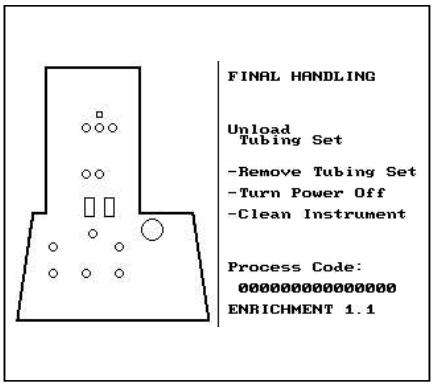


Fig. 4.4-1: Where to disconnect the tubing set after the run has been completed. Seal off above luer lock.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.4-4

Unload tubing set and shutdown

The window will display screen no. 4.4-4 as shown.

1. Remove the tubing set:
   Beginning with valve nos. 6, 9, 10 and 11, and working upwards, release the tubing from the liquid sensor and from the valves, by pressing on the valves. Release the columns from the column holders. Dispose the tubing set as a biohazard, according to standard hospital procedures.

2. Switch off the CliniMACS ^{plus} Instrument.

3. Clean the instrument according to cleaning instructions, see chapter 1.3.6. Follow the standard procedures for the treatment of infectious material.

Important

Prior to target cell infusion the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans. The user of the CliniMACS System is responsible for the selection of an appropriate medium for infusion.

If the volume of the target cell fraction exceeds 100 mL, it must be reduced by appropriate means, e.g. through centrifugation or buffer exchange to an infusion solution approved in your country.

Analysis of cells

The target cells must be examined regarding quality and quantity in view of their intended use. This must include the following parameters:

- Total number of leukocytes,
- Viability and total number of target cells,
- Purity and recovery of target cells.

It is also recommended to determine the total number of leukocytes and the viability of the non-target cell fraction.

The list is an example and other tests should be included based on the intended use and clinical protocols.

Record the analysis data.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

STEP 4:

ENRICHMENT 3.2

Once the final check has been completed, the CliniMACS plus Instrument is ready to begin the separation, the window will display screen no. 4.4-1 as shown.

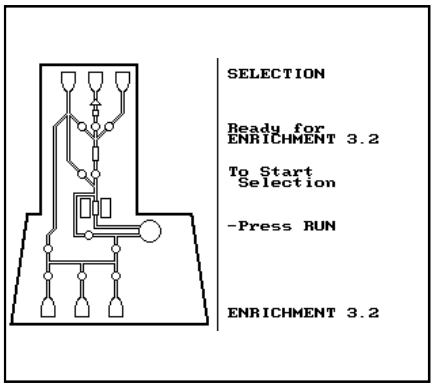
Make a final check of all tubing and attachments.

To proceed, press RUN

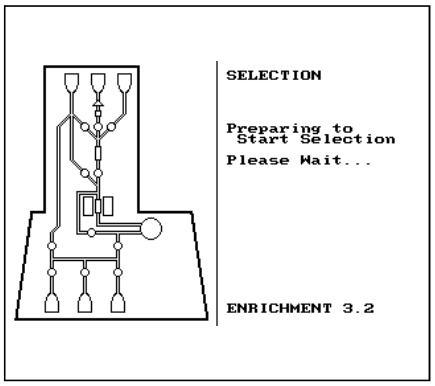
Once 'RUN' has been pressed, the instrument will automatically perform the separation procedure chosen.

At each phase of the operation, a screen similar to screen no. 4.4-2 is displayed to show the status of the separation procedure.

The magnet position indicator is displayed as two black boxes next to the separation column when the magnet is "ON",meaning it has been moved to the front to magnetize the separation column. If the magnet position indicator is transparent (e.g. screen no. 4.4-1), the magnet is "OFF", meaning it has been moved to the rear of the Instrument. With the magnet withdrawn the separation column is outside the magnetic field and is not magnetized.



Screen no. 4.4-1



Screen no. 4.4-2

Separation procedure

Loading cells

The separation procedure starts with the filling of the pre-system filter to complete the priming of the system. Buffer is pumped upwards towards the Cell Preparation Bag to fill the pre-system filter. Tap the side of the filter several times to remove any bubbles which might be trapped in the filter. Then, the separation of the labeled cells begins. The pump draws the contents of the Cell Preparation Bag into the tubing set. The magnetically labeled cells are retained in the separation column, placed in the magnetic field, while the unlabeled cells (non-target cells) are passed through and collected in the Negative Fraction Bag. When the Cell Preparation Bag is empty (detected automatically by the liquid sensor), the pre-system filter is rinsed twice with buffer.

Note

At each phase of the operation, the status of the separation procedure is shown on the screen.

Column wash I

The pre-column and separation column are washed extensively to remove all unlabeled cells. Wash buffer is collected in the Buffer Waste Bag.

Release of cells I

The magnet is moved to the rear of the instrument ("OFF" position). The retained cells are released at a high speed flow, but the cells remain within an internal tubing cycle.

Reloading of cells I

The magnet is moved into the "ON" position again to magnetize the separation column and the cells are reapplied onto the separation column.

Colum wash II

The reloading of the cells is followed by a second washing step to remove remaining unlabeled cells. Also, all tubing is rinsed several times.

Release of cells II, reloading of cells II, colum wash III

The cells are released and reapplied onto the separation column for a second time in order to remove any unlabeled cells sticking unspecifically to the column matrix. Afterwards the separation column is washed again.

Release of cells III, reloading of cells III, colum wash IV

Additionally, separation program ENRICHMENT 3.2 includes a third release and reapplication step.

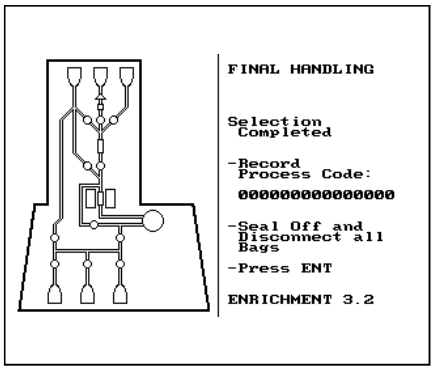
Final elution of cells

The magnet is moved into the "OFF" position and the magnetically labeled cells (target cells) are released and collected in the attached Cell Collection Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Disconnect bags and record process code

When the run has been completed, the CliniMACS plus Instrument will display screen no. 4.4-3 as shown.



Screen no. 4.4-3

1. Record the process code.

2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see fig. 4.4-1). Using the heat sealer, make three hermetic seals in the tubing directly below valve no. 9. Carefully sever the middle seal to disconnect the Cell Collection Bag from the tubing set.

3. Weigh the filled Cell Collection Bag and record the weight. Determine the weight of the target cell fraction by subtracting the weight of the empty Cell Collection Bag from the weight of the Cell Collection Bag containing the target cells. Record the weight.

4. Mix the target cell suspension thoroughly by rotating the bag. Take an aliquot of 0.5 mL and retain for analysis.

5. Using the heat sealer, seal off the tubing above the luer lock of the Negative Fraction Bag (see fig 4.4-1). Make three hermetic seals in the tubing. Sever the center seal to disconnect the Negative Fraction Bag.

6. Disconnect the Buffer Waste Bag in the same way (see fig. 4.4-1).

7. Remove the Negative Fraction Bag and Buffer Waste Bag. Keep all bags until analysis of all cells has been completed.

The target cells can now be processed in accordance with clinical protocols.

To proceed, press ENT

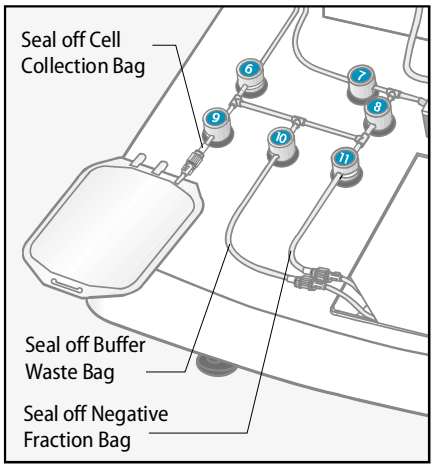
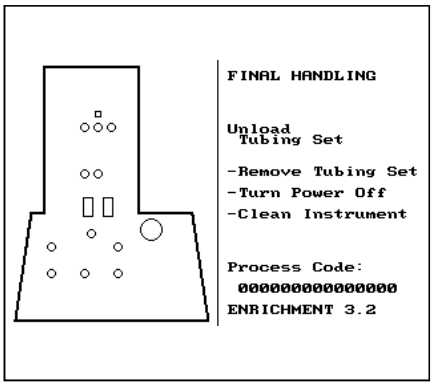


Fig. 4.4-1: Where to disconnect the tubing set after the run has been completed. Seal off above luer lock.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.4-4

Unload tubing set and shutdown

The window will display screen no. 4.4-4 as shown.

1. Remove the tubing set:
   Beginning with valve nos. 6, 9, 10 and 11, and working upwards, release the tubing from the liquid sensor and from the valves, by pressing on the valves. Release the columns from the column holders. Dispose the tubing set as a biohazard, according to standard hospital procedures.

2. Switch off the CliniMACS^{plus} Instrument.

3. Clean the Instrument according to cleaning instructions, see chapter 1.3.6. Follow the standard procedures for the treatment of infectious material.

Important

Prior to target cell infusion the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans. The user of the CliniMACS System is responsible for the selection of an appropriate medium for infusion.

If the volume of the target cell fraction exceeds 100 mL, it must be reduced by appropriate means, e.g. through centrifugation or buffer exchange to an infusion solution approved in your country.

Analysis of cells

The target cells must be analyzed regarding quality and quantity in view of their intended use.

This must include the following parameters:

- Total number of leukocytes,
- Viability and total number of target cells,
- Purity and recovery of target cells.

It is also recommended to determine the total number of leukocytes and the viability of the non-target cell fraction.

The list is an example and other tests should be included based on the intended use and clinical protocols.

Record the analysis data.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

STEP 4:

DEPLETION 2.1

Once the final check has been completed, the CliniMACS plus Instrument is ready to begin the separation, the window will display screen no. 4.4-1 as shown.

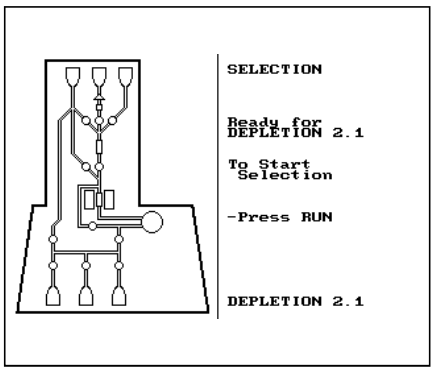
Make a final check of all tubing and attachments.

To proceed, press RUN

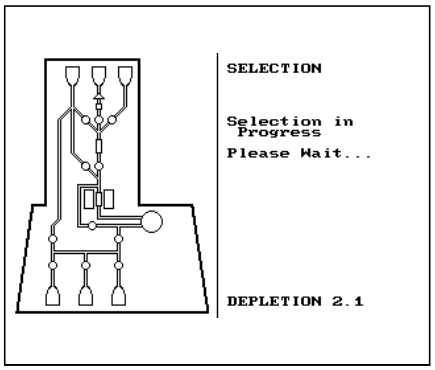
Once 'RUN' has been pressed, the instrument will automatically perform the separation procedure chosen.

At each phase of the operation, a screen similar to screen no. 4.4-2 is displayed to show the status of the separation procedure.

The magnet position indicator is displayed as two black boxes next to the separation column when the magnet is "ON", meaning it has been moved to the front to magnetize the separation column. If the magnet position indicator is transparent (e.g. screen no. 4.4-1), the magnet is "OFF", meaning it has been moved to the rear of the instrument. With the magnet withdrawn the separation column is outside the magnetic field and is not magnetized.



Screen no. 4.4-1



Screen no. 4.4-2

Separation procedure

Loading cells

The separation procedure starts with the filling of the pre-system filter to complete the priming of the system. Buffer is pumped upwards towards the Cell Preparation Bag to fill the pre-system filter. Tap the side of the filter several times to remove any bubbles which might be trapped in the filter. Then the separation of the labeled cells begins. The pump draws the contents of the Cell Preparation Bag into the tubing set. The magnetically labeled cells are retained in the separation column, placed in the magnetic field, while the unlabeled cells (target cells) are passed through and collected in the Cell Collection Bag.

Rinsing pre-system filter

After the cell sample has been completely loaded onto the tubing set detected by the liquid sensor, the pre-system filter is rinsed twice with buffer to reduce cell loss in the filter.

Note

At each phase of the operation, the status of the separation procedure is shown on the screen.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Note

- If the number of magnetically labeled cells (calculated by the CliniMACS Software) exceeds the binding capacity of the separation column, the separation program automatically loads and separates the cell sample in smaller portions ("staged loading").

- The loading is stopped when the capacity of the separation column is reached and the separation program then proceeds to the next step of the separation. After the last step (Final elution of the cells) finishes, the next portion of the sample is loaded onto the column. This sequence continues until the entire volume of magnetically labeled cells is loaded onto the separation column.

Column wash

The pre-column and separation column are washed extensively to remove all unlabeled cells. Wash buffer is collected in the Buffer Waste Bag.

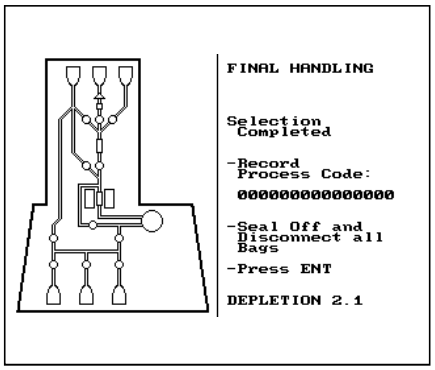
Final elution of the cells

The magnet is moved into the "OFF" position. At first, the magnetically labeled cells are eluted to the Priming Waste Bag while unlabeled cells (target cells) are collected in the Cell Collection Bag. Only after the last loading stage finishes are all magnetically labeled cells (non-target cells) released from the separation column and collected in the Negative Fraction Bag to reduce carryover with labeled cells in the pump tubing.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Disconnect bags and record process code

When the run has been completed, the CliniMACS plus Instrument will display screen no. 4.4-3 as shown.



Screen no. 4.4-3

1. Record the process code.

2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see fig. 4.4-1). Using the heat sealer, make three hermetic seals in the tubing directly below valve no. 9. Carefully sever the middle seal to disconnect the Cell Collection Bag from the tubing set.

3. Weigh the filled Cell Collection Bag. Record the weight. Determine the weight of the target cell fraction by subtracting the weight of the empty Cell Collection Bag from the weight of the Cell Collection Bag containing the target cells. Record the weight.

4. Mix the target cells suspension thoroughly by rotating the bag. Take an aliquot of 0.5 mL and retain for analysis.

5. Using the heat sealer, seal off the tubing above the luer lock of the Negative Fraction Bag (see fig 4.4-1). Make three hermetic seals in the tubing. Sever the center seal to disconnect the Negative Fraction Bag.

6. Disconnect the Buffer Waste Bag in the same way (see fig. 4.4-1).

7. Remove the Negative Fraction Bag and Buffer Waste Bag. Keep all bags until analysis of all cells has been completed.

The target cells can now be processed in accordance with clinical protocols.

To proceed, press ENT

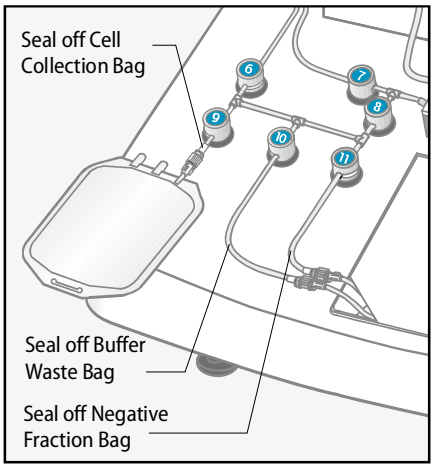
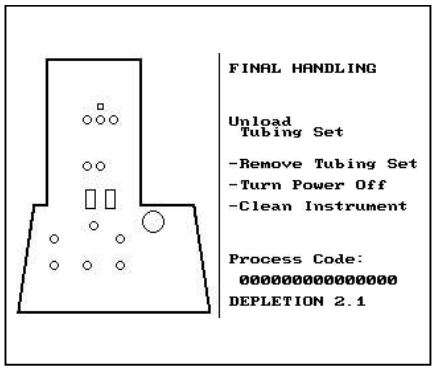


Fig. 4.4-1: Where to disconnect the tubing set after the run has been completed. Seal off above luer lock.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.4-4

Unload tubing set and shutdown

The window will display screen no. 4.4-5 as shown.

1. Remove the tubing set:
   Beginning with valve nos. 6, 9, 10 and 11, and working upwards, release the tubing from the liquid sensor and from the valves, by pressing on the valves. Release the columns from the column holders. Dispose the tubing set as a biohazard, according to standard hospital procedures.

2. Switch off the CliniMACS ^{plus} Instrument.

3. Clean the instrument according to cleaning instructions, see chapter 1.3.6. Follow the standard procedures for the treatment of infectious material.

Important

Prior to target cell infusion the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans. The user of the CliniMACS System is responsible for the selection of an appropriate medium for infusion.

If the volume of the target cell fraction exceeds 100 mL, it must be reduced by appropriate means, e.g. through centrifugation or buffer exchange to an infusion solution approved in your country.

Analysis of cells

The target cells must be examined regarding quality and quantity in view of their intended use. This must include the following parameters:

- Total number of leukocytes,
- Viability and total number of target cells,
- Purity and recovery of target cells.

It is also recommended to determine the total number of leukocytes and the viability of the non-target cell fraction.

The list is an example and other tests should be included based on the intended use and clinical protocols.

Record the analysis data.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

STEP 4:

DEPLETION 3.1

Once the final check has been completed, the CliniMACS plus Instrument is ready to begin the separation, the window will display screen no. 4.4-1 as shown.

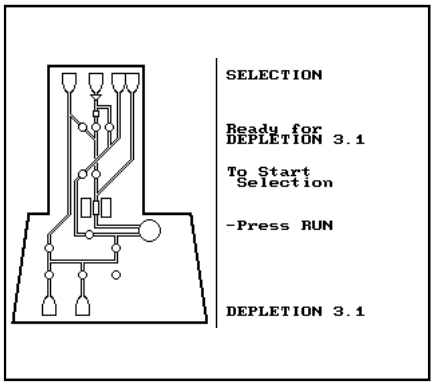
Make a final check of all tubing and attachments.

To proceed, press RUN

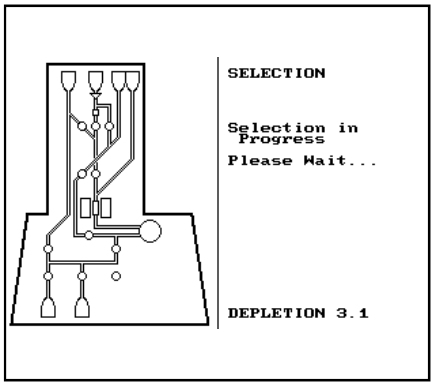
Once 'RUN' has been pressed, the instrument will automatically perform the separation procedure chosen.

At each phase of the operation, a screen similar to screen no. 4.4-2 is displayed to show the status of the separation procedure.

The magnet position indicator is displayed as two black boxes next to the separation column when the magnet is "ON", meaning it has been moved to the front to magnetize the separation column. If the magnet position indicator is transparent (e.g. screen no. 4.4-1), the magnet is "OFF", meaning it has been moved to the rear of the instrument. With the magnet withdrawn the separation column is outside the magnetic field and is not magnetized.



Screen no. 4.4-1



Screen no. 4.4-2

Separation procedure

The cells are applicated onto the separation column twice. In the first loading stages the bulk of labeled cells are depleted ("BULK LOADING STAGE"). In the second sensitive loading step the remaining labeled cells are depleted ("SENSITIVE LOADING STAGE").

Bulk loading stage

The separation procedure starts with the filling of the pre-aystem filter to complete the priming of the system. Buffer is pumped upwards towards the Cell Preparation Bag to fill the pre-system filter. Tap the side of the filter several times to remove any bubbles which might be trapped in the filter. Then the depletion of the labeled cells begins. The pump draws the contents of the Cell Preparation Bag into the tubing set. The magnetically labeled cells are retained in the separation column, placed in the magnetic field, while the unlabeled cells are passed through and collected in the Reapplication Bag.

Note

At each phase of the operation, the status of the separation procedure is shown on the screen.

Important

- If the number of magnetically labeled cells (calculated by the CliniMACS Software) exceeds the binding capacity of the separation column, the separation program automatically loads and separates the cell sample in smaller portions ("staged loading").

- Between the bulk loading and the sensitive loading stage, the separation column is washed to remove all labeled cells. The labeled cells are later eluted and held in the Non-Target Cell Bag. The wash buffer is collected in the Buffer Waste Bag.

Sensitive loading stage

For further depletion, the cells held in the Reapplication Bag are loaded onto the separation column again. The unlabeled cells (target cells) flow through the magnetic field and are collected in the Cell Collection Bag.

Rinsing pre-system filter and Reapplication Bag

After the cell sample has been completely loaded onto the tubing set, the pre-system filter and the Reapplication Bag are rinsed with buffer twice to reduce cell loss in the system.

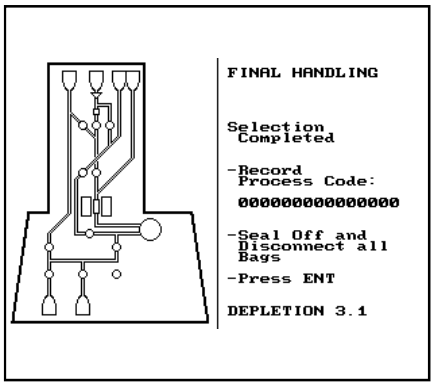
Final removal of non-target cells

The magnet is moved into the "OFF" position. The magnetically labeled cells (non-target cells) are released from the separation column and collected into the Non-Target Cell Bag.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Disconnect bags and record process code

When the run has been completed, the CliniMACS plus Instrument will display screen no. 4.4-3 as shown.



Screen no. 4.4-3

1. Record the process code.

2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see fig. 4.4-1). Using the heat sealer, make three hermetic seals in the tubing directly below valve no. 9. Carefully sever the middle seal to disconnect the Cell Collection Bag from the tubing set.

3. Weigh the filled Cell Collection Bag. Record the weight. Determine the weight of the target cell fraction by subtracting the weight of the empty Cell Collection Bag from the weight of the Cell Collection Bag containing the target cells. Record the weight.

4. Mix the target cell suspension thoroughly by rotating the bag. Take an aliquot of 0.5 mL and retain for analysis.

5. Using the heat sealer, seal off the tubing above the luer lock of the Buffer Waste Bag (see fig 4.4-1). Make three hermetic seals in the tubing. Sever the center seal to disconnect the Buffer Waste Bag.

6. Disconnect the Non-Target Cell Bag in the same way.

7. Remove the Non-Target Cell Bag and the Buffer Waste Bag. Keep all bags until analysis of all cells has been completed.

The target cells can now be processed in accordance with clinical protocols.

To proceed, press ENT

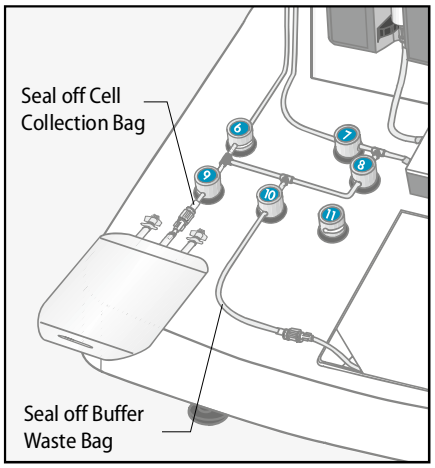
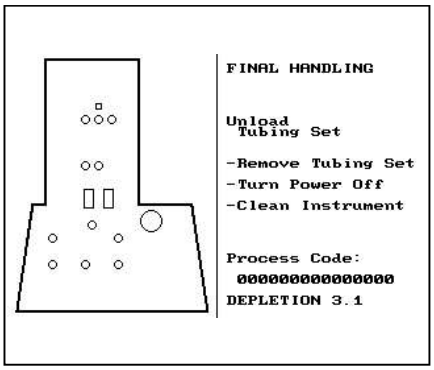


Fig. 4.4-1: Where to disconnect the tubing set after the run has been completed. Seal off above luer lock.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am



Screen no. 4.4-4

Important

Prior to target cell infusion the CliniMACS PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans. The user of the CliniMACS System is responsible for the selection of an appropriate medium for infusion.

If the volume of the target cell fraction exceeds 100 mL, it must be reduced by appropriate means, e.g. through centrifugation or buffer exchange to an infusion solution approved in your country.

Unload tubing set and shutdown

The window will display screen no. 4.4-4 as shown.

1. Remove the tubing set:
   Beginning with valve nos. 6, 9, and 10 and working upwards, release the tubing from the liquid sensor and from the valves by pressing on the valves. Release the column from the column holder. Dispose the tubing set as a biohazard, according to standard hospital procedures.

2. Switch off the CliniMACS ^{plus} Instrument.

3. Clean the instrument according to cleaning instructions, see chapter 1.3.6. Follow the standard procedures for the treatment of infectious material.

Analysis of cells

If the target cells are intended to be used for human applications, they must be examined regarding quality and quantity in view of their intended use. This must include the following parameters:

- Total number of leukocytes,
- Viability and total number of target cells,
- Purity and recovery of target cells.

It is also recommended to determine the total number of leukocytes and the viability of the non-target cells.

The list is an example and other tests should be included based on the intended use and clinical protocols.

Record the analysis data.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

5 Troubleshooting

This section is intended as a reference to provide information regarding possible unexpected events that might occur and to suggest appropriate corrective action. For information not covered in the following section, please contact a member of Miltenyi Biotec's Emergency Technical Support team by calling 1-800-810-3135 and selecting prompt number 1 as soon as possible.

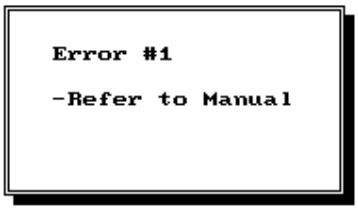
Please note that the order of this chapter follows the actual sequence of a separation.

5.1 Preparation of the leukapheresis product

The sample received is diluted.	Ideally, magnetic labeling is performed in diluted leukapheresis product. Adjust the leukapheresis product to a final dilution of approximately 1:3. If the sample received is diluted more than 1:3, or if the concentration of plasma in the sample is unknown, add immunoglobulin to the sample prior to the addition of the reagent (recommended concentration of immunoglobulin in the labeling volume: 1.5 mg/mL). It is important to include a certain amount of immunoglobulin in the sample during the labeling in order to minimize non-specific binding of the reagent.
The number of target cells is low in the leukapheresis product.	The mobilization of stem cells was insufficient. Check analysis of the leukapheresis product.
Poor viability of cells in the leukapheresis product.	The leukapheresis product may have been harvested, stored or transported inappropriately. To ensure better sample quality, the preparation and separation of the leukapheresis product should be performed immediately after leukapheresis. Keep the leukapheresis product at a leukocyte concentration of less than 0.2×10^9 per mL. If necessary, dilute the leukapheresis product with autologous plasma. The leukapheresis product should not be older than 24 hours when starting the labeling and separation procedure. If the leukapheresis product has to be stored, e.g. overnight, it should be kept at controlled room temperature (+19 °C to +25 °C).

5.2 CliniMACS® plus Instrument and CliniMACS Tubing Sets

Error messages



Error message no. 1

There are a number of possible instrument or software malfunctions. These are marked as such and will be displayed on the screen. They refer to internal errors that **cannot** be corrected by the operator. Record the displayed error number and contact a member of Miltenyi Biotec's Emergency Technical Support team.

One possible error message is shown in the illustration opposite (Error message no. 1).

Other than errror messages, malfuctions that can be corrected by the operator are marked "Warning messages". These are described in section 5.3.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Loading and priming of the tubing set

Valve does not open when operator is instructed to insert tubing into a particular valve.	The valves are designed to work properly once the tubing has been inserted. Press the valve manually to open it. Watch the valve carefully during the valve exercise sequence. If the valve does not depress during the valve exercise sequence, refer to the following event "Valve does not depress during valve exercise sequence".
Valve does not depress during valve exercise sequence.	Confirm that tubing is correctly inserted. Check whether valves have been cleaned thoroughly. Any valve that has been contaminated by fluid has to be exchanged. Please contact a member of Miltenyi Biotec's Emergency Technical Support team.
Buffer is leaking from tubing set during priming.	Tubing set is defective. Turn off the CliniMACS^{plus} Instrument and restart priming with a new tubing set installed and sufficient new buffer.
Excessive air occurs in tubing set after priming.	Buffer bag is not properly spiked. Use a new tubing set and sufficient new buffer and restart the CliniMACS run. Confirm that the septum of the buffer bag is properly punctured.
Unexpected volume of buffer in bags after priming. After priming, liquid should only be in the Priming Waste Bag and Buffer Waste Bag.	Tubing set is not mounted correctly. Liquid can leak behind the valves if the tubing set is not installed correctly or the valves are not functioning properly. Remove the tubing set and replace it with a new one. Restart the CliniMACS priming procedure with sufficient new buffer. Poor CliniMACS separation performance may result if the tubing set is not inserted properly.
Pump motor stalls during priming.	Pump tubing has not been inserted correctly. Press 'STOP' to interrupt the priming and turn the power "OFF" and then "ON" again. Clamp the buffer line with a haemostat during the installation procedure and remove the clamp before restarting the priming sequence.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

5.3 Automated cell separation

Warning messages

Unlike error messages (see section 5.2), warning messages are displayed on the screen when the internal control system of the CliniMACS plus Instrument recognizes a malfunction which **can** be corrected by the operator. Usually, a warning message appears in combination with a sound ("beep"). If a warning message appears during the CliniMACS run, follow the instructions on the screen to proceed with the cell separation. Generally speaking, warning messages appear when the 'STOP' key is pressed, when the pump door is opened, when the pump stalls or when the liquid sensor detects an error.

Since different kinds of unexpected events can occur during different separation programs, the following section has been subdivided accordingly.

Unexpected events – All separation programs

Error detected by liquid sensor.

Warning message no. 1

Warning message No. 1 will appear during the starting phase of the cell loading process if the liquid sensor is not able to detect liquid in the tubing. As the pre-system filter is rinsed with buffer prior to the cell loading, there must be liquid in the tubing at this point.

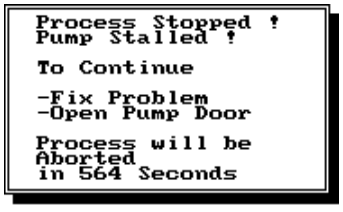
Check the following points:

1. Has the tubing been inserted correctly? If not, do so.
2. Is the tubing filled with buffer? If not, see point 3.
3. If the tubing is not filled with buffer, inspect the tubing for kinks blocking the buffer flow upwards into the pre-system filter and Cell Preparation Bag. Adjust the position of the tubing set. If necessary, raise or lower the bag hangers using the bag hanger clamps. Adjust the position of the tubing in the valves. To alter the position of the tubing, open the valve by manually pressing the button. Confirm that the tubing is not kinked, twisted or taut.
4. Is the Cell Preparation Bag spiked properly? Confirm the pre-system filter spike has penetrated the septum of the Cell Preparation Bag port.
5. If the tubing set has not been completely filled with buffer:
   Has the buffer spike of the tubing set penetrated the buffer bag?
   For correction see "Excessive air occurs in tubing set after priming" (section 5.2).

After the corrective action, continue with the separation in progress and press '5'.

If warning message no. 1 appears again after each of the possible causes listed above have been ruled out, the liquid sensor may be defective. Please contact a member of Miltenyi Biotec's Emergency Technical Support team.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Loading stopped before complete sample has been loaded onto the columns.	Liquid sensor defective or not filled correctly. Check liquid sensor by running the instrument check, taking care to insert the liquid filled tubing correctly into the liquid sensor. If the instrument check fails, contact a member of Miltenyi Biotec's Emergency Technical Support team.
Cells move to wrong part of tubing set. Liquid is leaking past valve(s).	- Tubing set has not been properly inserted. If the run is ongoing, press the 'STOP' and clamp the line with a hemostat. Adjust the tubing by first depressing the appropriate valve. Remove the clamp and press 'RUN' to resume separation. The cell separation will be aborted if 'RUN' is not pressed within 600 seconds. - Valve is not functioning properly. Press 'STOP'. Clamp the line with a hemostat. Depress the valve manually several times to un-stick the stuck valve. Remove the clamp and press 'RUN' to continue. The cell separation will be aborted if the 'RUN' key is not pressed within 600 seconds. If the user is unable to un-stick the valve, contact a member of Miltenyi Biotec's Emergency Technical Support team. - Wrong software program used. Check display for name of program currently used. Abort run by pressing the STOP key and immediately contact a member of Miltenyi Biotec's Emergency Technical Support team.
Magnet does not move.	Magnet drive does not work. - Due to an ongoing power failure, the magnet cannot be moved by the magnet drive. The viability of the cells trapped in the tubing set may be compromised. Contact a member of Miltenyi Biotec's Emergency Technical Support team. - A magnet drive failure has occurred. An error message will be displayed (see section 5.2, "Error messages"). Record the number of the error message and contact a member of Miltenyi Biotec's Emergency Technical Support team.
Pump motor stalls during cell separation.  Warning message no. 2	Pump tubing has not been inserted correctly, so the pump might be unable to rotate. In this case warning message no. 2 will appear on the screen window.The operator then has 600 seconds to correct the position of the pump tubing. 1. Carefully remove the pump tubing from the pump. 2. Confirm that the pump tubing has not been damaged by the incorrect insertion. If the pump tubing is leaking, clamp the tubing above and below the separation column to save the cells retained on the column and contact a member of Miltenyi Biotec's Emergency Technical Support team. 3. If the pump tubing has not been damaged, it can be reinserted into the pump housing.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

4. Press 'RUN' to restart the separation within 600 seconds or the separation will be aborted.

Sample loading does not stop although the Cell Preparation Bag and the pre-system filter are empty.

Liquid sensor is not working properly because the surface of the tubing in the liquid sensor maybe is wet. Press 'STOP' to interrupt the separation. Remove tubing from liquid sensor. Dry the tubing and the contact area using a paper towel or absorbent material. Replace the tubing in the liquid sensor and press 'RUN'. Do not interrupt the sequence for more than 600 seconds or the cell separation will be terminated.

If it is not possible to activate the liquid sensor following these steps, press 'STOP', then '2' and confirm with 'ENT' to skip sample loading and to continue the separation.

Unexpected events - CD34 SELECTION 1/2 and CD133 SELECTION 1/2

Loading stopped before complete sample has been loaded onto the columns.

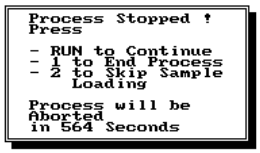
Pre-system filter is clogged due to large amount of cell debris or due to incomplete filling of the filter. Due to continued pumping, a vacuum was created which led to the generation of air bubbles activating the liquid sensor. Therefore, the separation process proceeded with the column washes before all of the sample could be loaded. It is not possible to restart the sample loading once the loading sequence has stopped.

Allow the CliniMACS plus Instrument to finish the separation program. Do not abort the current run, this may result in unnecessary cell loss. After the separation procedure finishes, filter the remaining sample with a 200 µm in-line blood filter and transfer it into a new transfer bag. Immediately perform a second separation with a new tubing set and sufficient new buffer.

Pump tubing collapsed and/or excessive air appeared in tubing set below pre-column during cell loading.

Pre-column is clogged due to large amount of cell debris in the Cell Preparation Bag. It is necessary to skip the loading of the remaining sample manually and to continue the separation with the cells that have already been loaded onto the system.

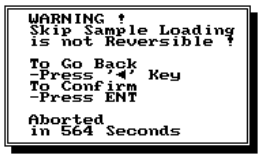
1. Press 'STOP' to interrupt cell loading. Warning message no. 3 will appear.



Warning message no. 3

2. Press '2' to skip the cell loading process.

   Warning message no. 4 will appear and will give the operator the opportunity to confirm or amend their decision. Because skipping of sample loading is not reversible.



Warning message no. 4

3. Press 'ENT' and the separation program will stop the sample loading and continue with column washes.

Clamp the tubing below the pre-system filter to prevent cells from leaking out of the Cell Preparation Bag into the tubing set. After the separation procedure has been finished, filter the remaining sample with a 200 µm in-line blood filter and transfer it into a new transfer bag. Immediately perform a second separation with a new tubing set and sufficient new buffer.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Unexpected events Enrichment programs only

Run is aborted before completion of cell separation program.

Warning message no. 5

- 'RUN' has not been pressed within 600 seconds after interrupting the procedure by pressing the 'STOP' key. The cell separation will not be completed. Recover as much of the sample as possible from the tubing set by running the EMERGENCY PROGRAM (see page 5 - 9).

- 'RUN' has not been pressed within 600 seconds after interrupting the procedure by opening the pump door. For safety reasons, the separation in progress will automatically stop during the instrument run if the pump door is opened. Message no. 5 will appear on the screen window.

  Close the pump door and press 'RUN'.

  If, 'RUN' is not pressed within 600 seconds, the separation will be aborted and the cell separation will not be completed. Recover as much of the sample as possible from the tubing set by running the EMERGENCY PROGRAM.

- Power failure results in the termination of the CliniMACS Separation. The cell separation will **not** be completed once the power supply has been restored. Recover as much of the sample as possible from the tubing set by running the EMERGENCY PROGRAM.

Unexpected events – ENRICHMENT 1.1

Loading stopped before complete sample has been loaded onto the columns.

Pre-system filter is clogged due to large amount of cell debris or due to incomplete filing of the filter. Due to continued pumping, a vacuum was created which has led to the generation of air bubbles. The air bubbles have activated the liquid sensor and the separation process continued with the final stage of sample loading. Some of the sample may remain in the Cell Preparation Bag after separation is finished.

Allow the CliniMACS ^{plus} Instrument to finish the separation program. Do not abort the current run, this may result in unnecessary cell loss.

Determine the total cell number, percentage of target cells, and the remaining sample loading volume in the Cell Preparation Bag, and enter actual sample parameters during set-up of the instrument. After the separation procedure has finished, filter the remaining sample with a 200 µm in-line blood filter and transfer it into a new transfer bag (Cell Preparation Bag). Immediately perform a second separation with a new tubing set and sufficient new buffer.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Wrong sample parameter values were entered.	The sample loading volume entered was lower than the actual volume in the attached Cell Preparation Bag. It is not possible to restart the sample loading once it has stopped. Allow the CliniMACS plus Instrument to finish the separation program. Do not abort the current run, this may result in unnecessary cell loss. Determine the total cell number, percentage of target cells, and the remaining sample loading volume in the Cell Preparation Bag and enter actual sample parameters during set-up of the instrument. Transfer the remaining sample into a new transfer bag (Cell Preparation Bag). Process remaining sample with a new tubing set, a new pre-system filter and sufficient new buffer.
Pump motor stalls during elution of target cells into Cell Collection Bag.	The clamp to the Cell Collection Bag is not open during the elution sequence. Therefore, the pathway to the Cell Collection Bag is blocked. Press 'STOP' and open the clamp to the Cell Collection Bag. Open the pump door and check whether the pump tubing is inserted correctly. Close the pump door and press 'RUN' to restart the elution sequence within 600 seconds.

Unexpected events – ENRICHMENT 3.2

Loading stopped before complete sample has been loaded onto the columns.	Pre-system filter is clogged due to large amount of cell debris or due to incomplete filling of the filter. Due to continued pumping, a vacuum was created which has led to the generation of air bubbles. The air bubbles have activated the liquid sensor and the separation process continued with the column washes before all of the sample was loaded. It is not possible to restart the sample loading once the loading sequence has stopped. Allow the CliniMACS plus Instrument to finish the separation program. Do not abort the current run, this may result in unnecessary cell loss. After the separation procedure has finished, filter the remaining sample with a 200 µm in-line blood filter and transfer it into a new transfer bag (Cell Preparation Bag). Immediately perform a second separation with a new tubing set and sufficient new buffer.
Pump motor stalls during elution of target cells into Cell Collection Bag.	The clamp to the Cell Collection Bag is not open during the elution sequence. Therefore, the pathway to the Cell Collection Bag is blocked. Press 'STOP' and open the clamp to the Cell Collection Bag. Open the pump door and check whether the pump tubing is inserted correctly. Close the pump door and press 'RUN' to restart the elution sequence within 600 seconds.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

EMERGENCY PROGRAM

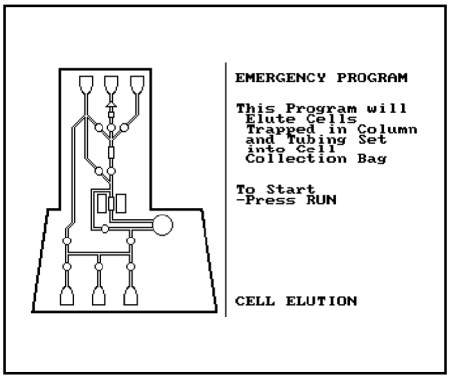
(to be used with enrichment programs only)

If, for any reason, a run has irreversibly terminated prior to the target cells' being eluted from the separation column, the EMERGENCY PROGRAM can be run to elute the cells from the separation column. This program has been designed only for use with all enrichment programs together with either a CliniMACS Tubing Set TS (REF 161-01) or a CliniMACS Tubing Set LS (REF 162-01). **The Emergency Program must not be used with any depletion programs.**

Note

- The emergency program will elute approximately 75 mL of fluid. Confirm that a suitable Cell Collection Bag is attached to the tubing set.

1. To confirm that the separation column is not magnetized, turn the instrument off, wait 5 seconds, then turn the instrument on again. The magnet is withdrawn from the magnetic separation unit (see Fig. 1.17, chapter 1.3.8). Check this by holding a small magnetizable item to the magnetic separation unit. If the magnet was not withdrawn, or if there is an ongoing power failure, please contact a member of Miltenyi Biotec's Emergency Technical Support team for help.
2. Wait until screen no. 1.2 appears in the window (see chapter 1.3.9).
3. To call up the EMERGENCY PROGRAM, press '4'.
4. Screen no. 5.1 appears:



Screen no. 5.1

To continue with the elution of the trapped cells, press 'RUN'.

5. Transfer the eluted cells collected in the Cell Collection Bag to a new 600 mL transfer bag. Eventually pool the remaining cells in the Cell Preparation Bag with the cells eluted by the Emergency Program. Start a new separation procedure using a new tubing set and sufficient new buffer.

Note

- To leave the EMERGENCY PROGRAM without starting the elution of the trapped cells, press 'STOP'.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Unexpected events – DEPLETION 2.1 (with TS LS)

Target cells do not reach the Cell Collection Bag.	Pump is unable to load the product from the Cell Preparation Bag because the clamp to the Cell Collection Bag is not open during the cell loading sequence. Press 'STOP' and open clamp to Cell Collection Bag. Continue the depletion process by pressing 'RUN'. Allow the CliniMACS plus Instrument to finish the separation program. Do not abort current run, this may result in unnecessary cell loss. If a part of the product remains in the Cell Preparation Bag after depletion procedure finished transfer the product to a new transfer bag (new Cell Preparation Bag) and, process the remaining cells with a new tubing set, a new presystem filter and sufficient new buffer. Determine the total cell number, percentage of labeled cells, and sample loading volume of the remaining product and enter actual sample parameters of the remaining product during the set-up of the instrument (STEP 2). Then begin the new depletion procedure.
Loading stopped before complete sample has been loaded onto the columns.	Air bubbles from the sample and/or pre-system filter activated the liquid sensor before all of the sample had been loaded. Abort the current run and check total cell number and depletion efficiency of the cells in the Cell Collection Bag. If necessary, process the remaining sample with a new tubing set, a new pre-system filter and sufficient new buffer. Determine the total cell number, percentage of labeled cells and sample loading volume and enter actual sample parameters during the set-up of the instrument (STEP 2) and start the new depletion of the remaining product.

Unexpected events – DEPLETION 3.1 (with DTS)

Loading stopped during **bulk loading stage** before complete sample has been loaded onto the columns.	Pre-system filter inserted wrong way around. Therefore, the drip chamber function is not available and air bubbles may pass the liquid sensor directly causing the termination of the sample loading before the complete sample was applied. The instrument continues the depletion process by immediately beginning the next step. It is not possible to restart the sample loading once it has stopped. Abort the current run and pool the contents of the Reapplication Bag and the Cell Preparation Bag. Process the remaining cells with a new tubing set, a new pre-system filter and sufficient new buffer.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Loading stopped during **sensitive loading stage.**	The Reapplication Bag is hanging lower than the Cell Preparation Bag. During sensitive loading step, Cell Preparation Bag runs empty before Reapplication Bag volume is completely loaded. It is therefore possible that an air bubble activates the liquid sensor before the sample is completely loaded from the Reapplication Bag after the depletion procedure is finished. It is not possible to restart sample loading once it has stopped. Allow the CliniMACS plus Instrument to finish the separation program. Do not abort current run, this may result in unnecessary cell loss. Process remaining sample with a new depletion tubing set, pre-system filter and sufficient new buffer. Determine the total cell number, percentage of labeled cells, and sample loading volume and enter actual sample parameters of the remaining product during the set-up of the instrument (STEP 2). Remember three large bag hangers are required for the program DEPLETION 3.1 and refer to installation instructions for appropriate bag heights (see chapter "STEP 3: CliniMACS Depletion Tubing Set").
Target cells (unlabeled cells) do not reach Reapplication Bag during bulk loading step.	Wrong tubing is inserted in valve no. 3 (right branch of Reapplication Bag tubing instead of left branch (Y-fitting) of tubing). Press 'STOP' and remove wrong tubing from valve no. 3. Manually insert correct tubing (left branch of Reapplication Bag tubing) into valve no. 3 as described in the installation instructions. Press 'RUN' to continue with the depletion procedure within 600 seconds. Allow the CliniMACS plus Instrument to finish the separation program. Do not abort the current run, this may result in unnecessary cell loss. Check depletion efficiency of the target fraction in the Cell Collection Bag. If depletion efficiency is not sufficient, repeat depletion procedure with a new tubing set, a new pre-system filter and sufficient new buffer. Determine the total cell number, percentage of labeled cells, and sample loading volume and enter actual sample parameter values during set-up of the instrument (STEP 2) and start the new depletion.

Cells flow into buffer bag during gravimetric rinsing steps.	Buffer bag is hanging too low (lower than Cell Preparation Bag). Press 'STOP' and adjust buffer bag hanger to correct position. Confirm that three large bag hangers are installed for the program DEPLETION 3.1 and refer to installation instructions for appropriate bag heights (see chapter "STEP 3: CliniMACS Depletion Tubing Set"). - If cells have not reached the buffer bag, it is sufficient to manually open valves nos. 1 and 2 to allow short backflushing of cells into the Cell Preparation Bag. After the buffer bag tubing is clear again, close valves nos. 1 and 2 and press 'RUN' to continue with the depletion procedure within 600 seconds. - If cells have already reached the buffer bag, exchange the buffer bag, manually open valves nos. 1 and 2 for a short flushing of the buffer bag tubing and press 'RUN' to continue with the depletion procedure within 600 seconds. Process the remaining sample in the buffer bag if necessary (if volume exceeds 300 mL, transfer the sample into a centrifugable bag for volume reduction first) using a new tubing set, a new pre-system filter, and sufficient new buffer.
Pump motor stalls during first elution of labeled cells into Non-Target Cell Bag.	- Hemostat was not removed after integrity test. Press 'STOP' and remove hemostat. Open pump door and check whether the pump tubing is correctly inserted. Close pump door and press 'RUN' to restart elution sequence within 600 seconds. - Wrong tubing was inserted in valve no. 3 (Non-Target Cell Bag tubing instead of left branch (Y-fitting) of Reapplication Bag tubing). Press 'STOP' and remove Non-Target Cell Bag tubing from valve no. 3. Manually insert correct tubing (left branch of Reapplication Bag tubing) into valve no. 3 as described in the installation instructions (see chapter "STEP 3: CliniMACS Depletion Tubing Set"). Press 'RUN' to restart the elution sequence within 600 seconds.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

5.4 Cell separation performance

Unexpected events – Enrichment programs only

The yield of target cells is low.

- Target cell content was over-estimated in the leukapheresis product. During analysis, target cells were incorrectly counted or an error occurred during counting of leukocytes. Repeat the analysis of leukapheresis product for starting target cell content.

- Target cells were poorly labeled with the reagent.
  - Reagent has expired. Check use-by date. Do not use any reagent after the use-by date.
  - Reagent was not stored properly. Check storage temperature. Do not use any reagent that was stored improperly (see reagent package insert).
  - Recommended labeling procedure was not followed. Refer to sample preparation and separation procedure chapters in the manual.

- Cells were lost during the preparation steps.
  - Cells were removed with the supernatant into Plasma Waste Bag and Wash Waste Bags due to incomplete sedimentation or too early resuspension of the cells, e.g. when the bag was removed from the centrifuge. Compare leukocyte content of the unlabeled leukapheresis product and the labeled leukapheresis product. Check centrifugation settings for proper centrifugation. Determine cell counts from all waste bags.
  - Buffer did not contain HSA. Supplement the buffer with HSA to a final concentration of 0.5 % (w/v), (see chapter "Preparation of CliniMACS PBS/EDTA Buffer", STEP 1).
  - Centrifuge settings were suboptimal. Check centrifugation settings.
  - Centrifuge imbalance or use of brake or asymmetrical loading of centrifuge.

- Cell viability decreased during preparation. See section "Viability of the positive fraction is less than 90% or the color of the supernatant during the washing steps was red" below.

- Analysis was incorrect.
  - Sampling error occured. Check cell suspension for clumped or settled cells. Confirm that representative samples are taken and repeat analysis.
  - Staining error occured. Check flow cytometry reagents. Repeat staining.
  - Flow cytometer settings were improper. Check instrument settings.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

The purity of target cells is low.	- The leukapheresis product was stored inappropriately. Preparation and separation of the leukapheresis product should be performed immediately after leukapheresis. Keep the leukapheresis product at a leukocyte concentration of less than 0.2×10^9 per mL. If necessary, dilute the leukapheresis product with autologous plasma. The leukapheresis product should not be older than 24 hours when starting the labeling and separation procedure. If the leukapheresis product must be stored, e.g. overnight, it should be kept at controlled room temperature (+19 °C to +25 °C). - The magnetic labeling protocol was not followed (e.g., incorrect volumes during magnetic labeling). Please follow the instructions given for the magnetic labeling (STEP 1). For troubleshooting purposes determine the leukocyte subsets (B cells, T cells, monocytes, granulocytes as well as platelets) contaminating the target fraction and contact a member of Miltenyi Biotec's Emergency Technical Support team for advice. - High numbers of granulocytes contaminated the cellular starting product (suboptimal apheresis setting). Dying granulocytes will then bind the CliniMACS Reagent non-specifically which may lead to decreased purity of the target cells. - Valve malfunction occurred. Eluted target cell fraction was contaminated by part of the negative fraction or buffer waste fraction. Inspect tubing placement within the valves to ensure proper functioning. Assess target cell content of the negative fraction and buffer waste. If necessary, pool the target and negative fraction, reduce to suitable volume and repeat the run with a new tubing set and sufficient new buffer.
Viability of the positive fraction is less than 90% or the color of the supernatant during the washing steps was red.	Cell lysis occurred due to incorrect osmolarity of the buffer. Check buffer and use recommended buffer (see CliniMACS PBS/EDTA Buffer, 1.4 Glossary).
Non-specific retention of dead cells from leukapheresis product or high non-specific cell losses throughout the procedure.	- Buffer does not contain HSA. Supplement the buffer with HSA to a final concentration of 0.5 % (w/v), (see "Preparation of CliniMACS PBS/EDTA Buffer", STEP 1). - The leukapheresis product may have been stored inappropriately. Preparation and separation of the leukapheresis product should be performed immediately after leukapheresis. Keep the leukapheresis product at a leukocyte concentration of less than 0.2×10^9 per mL. If necessary, dilute the leukapheresis with autologous plasma. The leukapheresis product should not be older than 24 hours when starting the labeling and separation procedure. If the leukapheresis product must be stored, e.g. overnight, it should be kept at controlled room temperature (+19 °C to +25 °C). - Incomplete sample loading due to clogging of separation column, pre-system filter, or pre-column. Check total cell number and depletion efficiency of the remaining cells.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

InfoCard #: STCL-PROC-015 JA6 Rev. 01 Effective Date: 28 Jun 2023

Unexpected events – CD34 SELECTION 1/2, CD133 SLELECTION 1/2 and ENRICHMENT 1.1

The purity of the target cells is low.

Elution from the separation column was incomplete.

- Separation program was aborted. Check display screen for error message. Continue with section "Run is aborted before completion of cell separation program" (see section 5.3).
- Pump failure or valve failure occured. Recover cells from the tubing set following the EMERGENCY PROGRAM described in section 5.3. Check volumes of all fractions. Assess target cell content of Buffer Waste Bag and Negative Fraction Bag.
- Tubing to Cell Collection Bag is blocked. Check tubing set for closed clamps, occlusions or kinks.
- Tubing has not been properly inserted. Check all valves for proper tubing insertion.

Unexpected events – CD34 SELECTION 1/2 and CD133 SELECTION 1/2

The purity of the target cells is low.

Non-target cells were retained.

- Mobilization of target cells was poor. Very low number of target cells has occurred. Therefore, a low number of contaminating non-target cells (e.g. granylocytes, monocytes, platelets) may lead to decreased purity.
- Insufficient plasma or immunoglobulins were present during magnetic labeling. Please follow the instructions provided in STEP 1 regarding magnetic labeling of the product.

  If a final concentration of about 30% autologous plasma in the sample during magnetic labeling cannot be guaranteed, add immunoglobulin to the sample. A final concentration of 1.5 mg/mL is recommended for the efficient blocking of non-specific reagent binding during magnetic labeling.

Unexpected events – ENRICHMENT 1.1

The purity of the target cells is low.

Incorrect sample parameter input. Cross-check parameter input with analysis results.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Unexpected events – DEPLETION 2.1 and DEPLETION 3.1

The depletion efficiency is low.	If the depletion efficiency is insufficient, process the remaining sample with a new depletion tubing set and sufficient new buffer. A new labeling of the cells targeted for depleted should be considered. Determine the cell count and percentage of cells for depletion and enter actual sample parameters during set-up of the instrument (STEP 2). If visible clumps occur, it may be helpful to filter the cells prior to a new run. Take into account that cells may be lost due to clumping and additional filtration. - Capacity of reagent and/or tubing set has been exceeded. Refer to capacity limits of the relevant products. - Incorrect determination of cells to be labeled by the reagent. - Incorrect sample parameter input. Cross-check parameter input with analysis results. - Reduced viability of the cells. Dead cells can bind non-specifically to the separation column reducing the labeling capacity of the column, and potentially resulting in lower depletion efficiency. Ensure better leukapheresis product quality and process product as fresh as possible. Stored products that are older than 24 hours should not be used. If storage is necessary, leukapheresis product should be kept at controlled room temperature (+19 °C to +25 °C). The cell concentration should not exceed 0.2×10^9/mL. If necessary, dilute the leukapheresis product with autologous plasma to achieve optimal cell concentration.

CONFIDENTIAL - Printed by: CWC35 on 10 Jul 2023 11:01:11 am

Signature Manifest

Document Number: STCL-PROC-015 JA6 **Revision:** 01
Title: CliniMACS User Manual US Edition JA6
Effective Date: 28 Jun 2023

All dates and times are in Eastern Time.

STCL-PROC-015 JA6 CliniMACS User Manual US Edition JA6

Author

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATER002)		26 Jun 2023, 11:45:00 AM	Approved

Management

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATER002)		26 Jun 2023, 11:45:20 AM	Approved

Medical Director

Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		26 Jun 2023, 02:44:18 PM	Approved

Quality

Name/Signature	Title	Date	Meaning/Reason
Melissa Ritt (MSR68)		27 Jun 2023, 09:44:31 AM	Approved

Document Release

Name/Signature	Title	Date	Meaning/Reason
Sandra Mulligan (MULLI026)		27 Jun 2023, 10:31:54 AM	Approved