



STEM CELL LABORATORY (STCL)



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Thawing of Cryopreserved Cellular Products in Preparation for CD34 Selection Using the Miltenyi CliniMACS

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THAWING OF CRYOPRESERVED CELLULAR PRODUCTS IN PREPARATION FOR CD34 SELECTION USING THE MILTENYI CLINIMACS

1 PURPOSE

- 1.1 This document describes the procedure for thawing cryopreserved apheresis products and the preparation of the thawed product for the selection and enrichment of human CD34 positive hematopoietic progenitor cells utilizing the CliniMACS magnetic cell separation system.

2 INTRODUCTION

- 2.1 The CliniMACS system is a fully automated device designated for cell processing and selection. It is used for the positive selection of CD34 positive cells or for simultaneous CD34 positive cell selection with purging of unwanted cells from a heterogeneous cell population. The CliniMACS system utilizes highly specific CD34 monoclonal antibodies conjugated to super-paramagnetic particles. Super-paramagnetic particles are small in size (about 50nm in diameter) and are composed of iron oxide and dextran. The magnetic particles form a stable colloidal suspension and do not precipitate nor aggregate in magnetic fields. The CD34 positive cells are specifically labeled for selection during incubation with the CliniMACS CD34 Reagent. After unbound reagent is washed from the suspension, the cells are ready to be selected in the automated continuous flow separation system. The CliniMACS system passes the antibody labeled cell suspension through a column in which strong magnetic gradients are generated. The targeted cells are retained in the selection column, where they are washed several times to remove any extraneous material. After the final wash, the column is removed from the magnetic field and the targeted cells are eluted to the collection bag.
- 2.2 The addition of MgCl is needed to catalyze the DNase reaction. Adding IgG during the labeling phase helps to reduce non-specific binding of the reagent. It is recommended to use only the LS Tubing set no matter what the cell load (the LS tubing set can be used whether one or two vials of reagent are required for the incubation as determined by the number of TNC and target cells) as it provides the capability of a higher dilution of the product to be loaded (275 mL).
- 2.3 When processing cellular products for transplantation, sterile technique should be used whenever feasible. Every precaution should be taken to minimize the possibility of contaminating cellular products.

3 SCOPE AND RESPONSIBILITIES

- 3.1 The Medical Directors, Laboratory Manager, Quality System Unit, and applicable laboratory staff are responsible for ensuring that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

- | | | |
|------|------|---|
| 4.1 | HSA | Human Serum Albumin |
| 4.2 | ACDA | Anticoagulant Citrate Dextrose Solution USP Formula A |
| 4.3 | MgCL | Magnesium Chloride |
| 4.4 | IgG | Immune Globulin |
| 4.5 | M | Molar |
| 4.6 | BSC | Biological Safety Cabinet |
| 4.7 | SOP | Standard Operating Procedure |
| 4.8 | mL | milliliter |
| 4.9 | mg | milligram |
| 4.10 | G | gauge |
| 4.11 | QC | Quality Control |
| 4.12 | HPCA | Hematopoietic Progenitor Cell Assay |
| 4.13 | RPM | Revolutions per Minute |
| 4.14 | RBC | Red Blood Cell |
| 4.15 | ° | Degree |
| 4.16 | C | Celsius |

5 MATERIALS

- 5.1 Plasmalyte A (1000 mL bags)
- 5.2 HSA
- 5.3 ACDA
- 5.4 Pulmozyme (2.5 mL @ 1mg/mL = 1000 units/mL)

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Stem Cell Laboratory, DUMC
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- 5.5 MgCl (Stock 1M)
- 5.6 Immune Globulin Intravenous (Human) GAMMAGARD S/D
- 5.7 Sample Site Coupler
- 5.8 Syringes (1 mL, 5 mL, 10 mL, 20 mL, 30 mL, 60 mL)
- 5.9 Sterile needles; 16G or 19G
- 5.10 Alcohol prep pads
- 5.11 Transfer pack(s) (300 mL, 600 mL, 1000 mL)
- 5.12 Blood Filter (170 microns)
- 5.13 Sterile Tubing Welding Wafers

6 EQUIPMENT

- 6.1 37 °C Waterbath
- 6.2 Temperature controlled centrifuge
- 6.3 Plasma extractor
- 6.4 Biological safety cabinet
- 6.5 Sterile Tubing Welder
- 6.6 Balance
- 6.7 Tubing heat sealer
- 6.8 Tubing stripper
- 6.9 Hemostats
- 6.10 Timer

7 SAFETY

- 7.1 Use all appropriate personal protective equipment (PPE) when handling any/all potentially hazardous blood and body fluids to include, but not limited to, gloves, lab coats, goggles, etc.

8 PROCEDURE

- 8.1 All work in this procedure should be performed in a BSC whenever possible using aseptic technique at all times.
- 8.2 Record lot numbers and expiration dates for all appropriate reagents and disposables on the worksheet (*STCL-PROC-015 (FRM1) CliniMACS Worksheet*) and/or on the appropriate lot sheet (*STCL-FORM-044 Processing Lot Numbers – 37 °C Thaw*).
- 8.3 Use an apheresis product (or combination of) with at least 3×10^8 CD34+ total.
- 8.4 Ensure centrifuge is at room temperature.
- 8.5 Prepare the following reagents:
 - 8.5.1 **Thaw Buffer:** (1) 1000 mL bag Plasmalyte A at 4 °C (remove 60 mL); 1% HSA (add 250 mL 5% HSA or 50 mL 25% HSA); 60 mL ACDA (replaces NaCitrate)
 - 8.5.2 **DNase Wash Buffer:** (2) 1000 mL bags Plasmalyte A; 0.5% HSA (add 111 mL 5% HSA or 22.2 mL 25% HSA); 5 vials Pulmozyme; 0.25 mL MgCl (stock 1M or 0.5 mL with x2 dilution)
 - 8.5.3 **CliniMACS Run Buffer:** (1) 1000 mL bag Plasmalyte A (remove 100 or 20 mL dependent on HSA concentration); 0.5% HSA (add 100 mL 5% HSA or 20 mL 25% HSA); 4 vials of Pulmozyme; 0.21 mL MgCl (stock 1M)
- 8.6 Thaw cells in a 37 °C waterbath to a “slushy” consistency, leaving small amounts of ice.
- 8.7 Transfer all bags to a BSC, aseptically clean the port(s) and spike each bag with a sample coupler.
- 8.8 Immediately inject Pulmozyme (1000 units/mL) at 10 units/mL of product (ie. If have 50 mL of product, inject 0.5 mL Pulmozyme).
- 8.9 Immediately inject 0.125 mL of stock 1M MgCl (0.5 mL/10 mL or 0.1 mL/10 mL of diluted 1:2 1M stock solution)
- 8.10 For each thawed cryobag, using a 60 mL syringe, withdraw contents and transfer to a 600 mL transfer pack.
- 8.11 Rinse each bag with 50 mL of Thaw Buffer and transfer to same 600 mL transfer pack.
- 8.12 Remove a sample to perform initial pre-processing QC (Sample “A”) to include cell count, trypan blue viability, manual differential, HPCA and flow analysis; record results on laboratory worksheet.

- 8.13 Fill the 600 mL transfer pack containing the thawed product/rinses to about 600 mL with Thaw Buffer.
- 8.14 If noticeable clumps are present, insert standard blood filter, filter the product and transfer to another 600 mL transfer pack.
- 8.15 Centrifuge at 300 g for 15 minutes at room temperature, no brake.
- 8.16 Express supernatant and gently and thoroughly mix cell pellet.
- 8.17 Dilute with DNase Wash Buffer to about 600 mL.
- 8.18 If noticeable clumps are present, insert standard blood filter, filter the product and transfer to another 600 mL transfer pack.
- 8.19 Centrifuge at 300 g for 15 minutes at room temperature, no brake.
- 8.20 Express supernatant, gently mix cell pellet and adjust volume to target volume with fresh DNase Wash buffer.
 - 8.20.1 Target volume equals 95 mL +/- 5 mL if using 1 vial of CD34+ reagent (for less than 60×10^9 TNCC).
 - 8.20.2 Target volume equals 190 mL +/- 5 mL if using 2 vials of CD34+ reagent (for $60 - 120 \times 10^9$ TNCC).
- 8.21 Calculate the desired amount of IgG to add to obtain a product concentration of 1.5 mg/mL. Add calculated amount to target volume.
 - 8.21.1 IgG is ordered through the pharmacy or directly from Baxter: Immune Globulin Intravenous (Human) GAMMAGARD S/D 5g, 96 mL, store at a temperature not to exceed 25° C.
 - 8.21.2 Allow IgG to reach room temperature before reconstitution if refrigerated.
 - 8.21.3 To reconstitute a 10% solution of IgG, remove 48 mL from the 96 mL of the included sterile water diluent and discard.
 - 8.21.4 Add the remaining 48 mL of sterile water diluent to the concentrate bottle and immediately swirl the concentrate bottle gently to thoroughly mix contents.
 - 8.21.5 Rotate gently until all concentrate is dissolved. Do NOT shake to avoid foaming.

NOTE: Use within 24 hours of reconstituting and then discard any remaining solution.
 - 8.21.6 Add 1.5 mg human IgG per mL of cellular product.
 - 8.21.6.1 If target volume for addition of CD34 reagent is 95 mL, multiply 1.5 mg times 95 mL to determine that 142.5 mg of IgG is needed.

8.21.6.2 In a 5g bottle, there are 5000 mg reconstituted in 48 mL.
Use a proportion to calculate the desired volume needed.

$$\frac{5000 \text{ mg}}{48 \text{ mL}} = \frac{142.5 \text{ mg}}{x} \quad x = 1.4 \text{ mL of IVIg needed}$$

- 8.22 Add about a 50 mL air pocket.
- 8.23 Mix gently and incubate at room temperature for 5 minutes.
- 8.24 Add the determined number of CliniMACS CD34+ reagent vials to the product and mix gently.
- 8.25 Incubate at room temperature for 30 minutes, rotating and inverting the bag every 5 minutes.
- 8.26 If clumping is noted, filter to a new 600 mL transfer pack and rinse filter by injecting about 50 mL of DNase Wash Buffer into the empty/drained transfer pack.
- 8.27 Dilute with DNase Wash Buffer to about 600 mL.
- 8.28 Centrifuge at 300 g for 15 minutes at room temperature, no brake.
- 8.29 Express the supernatant and gently mix cell pellet.
- 8.30 Again, dilute with DNase Wash Buffer to about 600 mL.
- 8.31 Centrifuge at 300 g for 15 minutes at room temperature, no brake.
- 8.32 Express the supernatant and gently mix cell pellet, resuspend pellet with DNase Wash Buffer to target volume of 275 mL for use with the LS tubing set.
- 8.33 If clumping is noted, filter to a new 600 mL transfer pack.
- 8.34 In BSC, add about 50 mL of air to the product.
- 8.35 Remove a sample to perform pre-CliniMACS, post incubation/wash QC (Sample "B") to include cell count, trypan blue viability, HPCA and flow analysis; record results on laboratory worksheet.
- 8.36 Product is ready to process on CliniMACS instrument, return to *STCL-PROC-015 CD34 Positive Selection using Miltenyi CliniMACS*, step 8.20 to proceed with selection of cellular product.

NOTE: Select "CD34 Selection 2" program when using the LS tubing set. Substitute CliniMACS Run Buffer, prepared in step 8.5.3, for CliniMACS PBS/EDTA buffer.

9 RELATED FORMS

- 9.1 STCL-PROC-015 FRM1 CliniMACS Worksheet
- 9.2 STCL-PROC-015 FRM2 CD34+ Certificate of Analysis (COA)
- 9.3 STCL-PROC-015 FRM3 CD34+ Certificate of Analysis (COA) for CliniMACS Allogeneic Donors
- 9.4 STCL-PROC-015 JA1 Auto CD34+ Selected PBPC Treatment of Severe Chron's Disease General Practices for Miltenyi CliniMACS
- 9.5 STCL-PROC-015 JA2 Preparation of Bone Marrow before CD34+ Selection Procedure
- 9.6 COMM-PAS-003 Labeling Cellular Therapy Products
- 9.7 STCL-FORM-044 Processing Lot Numbers – 37 °C Thaw
- 9.8 STCL-FORM-049 Processing Lot Numbers – Incoming Cellular Product Processing

10 REFERENCES

- 10.1 CliniMACS System User Manual, Miltenyi Biotec, Presidential Way, Woburn, Massachusetts, 01801.
- 10.2 Adult Bone Marrow Transplant Program Protocol Notebooks – internal protocols, Duke University Medical Center, Durham, NC.

11 REVISION HISTORY

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