



STEM CELL LABORATORY (STCL)



DOCUMENT NUMBER: STCL-PROC-019 FRM1

DOCUMENT TITLE:

CD56+ Selection Worksheet FRM1

DOCUMENT NOTES:

Document Information

Revision: 04

Vault: STCL-Processing-rel

Status: Release

Document Type: STCL

Date Information

Creation Date: 14 Jun 2021

Release Date: 06 Jul 2021

Effective Date: 06 Jul 2021

Expiration Date:

Control Information

Author: WATER002

Owner: WATER002

Previous Number: STCL-PROC-019 FRM1 Rev (**Change Number:** STCL-CCR-521

**STCL-PROC-019 FRM1
CD56+ SELECTION CLINIMACS WORKSHEET**

BARCODE*

Recipient Name: _____ **History #:** _____ **ABO/Rh:** _____

Donor Name: _____ **History #:** _____ **ABO/Rh:** _____

(* In the event that products are pooled to obtain a sample for this selection procedure, affix the barcodes of each of the pooled products below, and assign a new barcode to designate the pooled product.)

BARCODE

BARCODE

Recipient's Weight (kg): _____

Product Description: _____

Date of Procedure: _____

Performing Technologist: _____

Product/Procedure Limitations:

- $\leq 10 \times 10^9$ CD56+ cells; $\leq 40.0 \times 10^9$ TNC: 1 vial CliniMACS CD56 reagent
- $\leq 5.0 \times 10^9$ CD56+ cells; $\leq 40.0 \times 10^9$ TNC: Tubing Set Ref No. 161-01
- $\leq 2.0 \times 10^9$ CD56+ cells: 2 liters prepared PBS/EDTA buffer; 300 mL cell collection bag
- $2 - 5.0 \times 10^9$ CD56+ cells: 3 liters prepared PBS/EDTA buffer; 600 mL cell collection bag

Pre-Overnight Testing (if applicable)

Remove sample for cell count, viability, flow, ABO, sterility, and nuncs: _____

Product Cell Count ($\times 10^6$): _____

Product Volume (mL): _____

Total Cells ($\times 10^9$): _____

Viability: _____

Sterility Bottles Inoculated: _____

Nuncs: _____

BARCODE*

Pre-Sample Testing (Sample A)

Remove sample for cell count, viability, flow, and HPCA. If no pre-overnight testing performed, also remove samples for ABO, sterility, and nuncs: _____

Product Cell Count ($\times 10^6$): _____ Product Volume (mL): _____

Total Cells ($\times 10^9$): _____ Viability: _____

Sterility Bottles Inoculated: _____ Nuncs: _____

Sample taken to Flow and HPCA: _____

Working Buffer Prepared (20 mL of 25% HSA to each liter of PBS/EDTA buffer): _____

- 2 liters for products with $\leq 2.0 \times 10^9$ CD56+ cells
- 3 liters for products with $2 - 5.0 \times 10^9$ CD56+ cells

Wash I

Transfer well mixed product to a labeled 600 mL transfer pack and weigh to obtain volume: _____ grams

Volume of product subtracted from 600: _____ grams (amount of buffer to add)

Actual amount of PBS added: _____ grams

Total volume of product and buffer: _____ grams

Centrifuge at room temperature at 1250 RPM for 15 minutes, **no brake**: _____

Remove from centrifuge, let hang 10 minutes, and express **all** supernatant:

- Volume expressed: _____ grams
- Volume remaining in cell bag: _____ grams
- Volume of buffer to add: _____ grams
 - Target volume = 95 +/- 5 mL (95grams-106 grams)
- Final volume: _____ grams (divide by 1.06 to convert to mL) _____ mL

Mix cells gently and thoroughly: _____

Cell Processing – Labeling

Inject **cold** CD56+ reagent (followed by a syringe of air): _____

Amount of reagent injected: _____

Time reagent injected: _____

Incubate for 30 minutes at room temperature with gentle rotating/mixing every 5 minutes: _____

BARCODE*

Wash II

Weigh to obtain volume: _____grams

Volume of product subtracted from 600: _____grams, (amount of buffer to add)

Actual amount of PBS added: _____grams

Total volume of product and buffer: _____grams

Centrifuge at room temperature at 1250 RPM for 15 minutes, **no brake**: _____Remove from centrifuge, let hang 10 minutes, and express **all** supernatant:

- Volume expressed: _____grams
- Volume remaining in cell bag: _____grams
- Volume of buffer to add: _____grams
 - Target volume = 150 +/- 5 mLs (154 grams to 164 grams)
- Final volume: _____grams (divide by 1.06 to convert to mL) _____mL.

Mix cells gently and thoroughly: _____

Pre-CliniMACS Testing (Sample B)

Remove 1.0 mL for cell count, viability, flow, and HPCA: _____

Product Cell Count ($\times 10^6$): _____

Product Volume (mL): _____

Total Cells ($\times 10^9$): _____

Viability: _____

Cell Processing – Magnetic Separation (CliniMACS)

In the BSC, inject 60 mL of air to product bag prior to connecting to tubing set: _____

Insert a Plasma Transfer Set with a female luer adapter into a labeled transfer pack: _____

- 300 mL transfer pack for products with $\leq 2.0 \times 10^9$ CD56+ cells
- 600 mL transfer pack for products with $2 - 5.0 \times 10^9$ CD56+ cells

Weigh the pack/transfer set and record weight (tare weight for the final product): _____

Unpack the tubing set under the hood, tighten the connectors, and attach the cell collection bag to the luer connector on the tubing set: _____

- Tubing Set Ref No. 161-01 for products with $\leq 5.0 \times 10^9$ CD56+ cells

Connect the pre-system filter to the tubing set:

- Remove the cap from the bubble trap spike of the drip chamber: _____
- Remove the cap from the lower opening (non-spike) of the pre-system filter and firmly insert the spike into the pre-system filter: _____
- **NOTE**: Be extremely careful; refer to STCL-PROC-018 for further connection instructions.

BARCODE*

Connect the cell preparation bag to the pre-system filter:

- Clamp the tubing below the bubble trap: _____
- Remove the cap from the pre-system filter spike and spike the cell preparation bag: _____

Connect the remaining buffer bag to the tubing set:

- Clamp the tubing below the spike on the tubing set: _____
- Remove the cap from the tubing set spike and spike the buffer bag: _____
- **NOTE:** If $2 - 5 \times 10^9$ CD56+ cells, the CliniMACS will require 2 liters of prepared working buffer. Connect these using a plasma transfer set, sampling site coupler, and 16G needle. Be sure to close the roller clamp on the plasma transfer set **before** spiking into the buffer bag.

Set up the CliniMACS:

- Select ENRICHMENT 1.1: _____
- Select CLINIMACS TS for tubing set Ref. No. 161-01: _____
- WBC concentration (Sample B): _____
- Percentage of labelled cells (% viable CD56+ [Total] on Sample A flow printout): _____
- Sample loading volume (Sample B): _____
- Follow remaining steps on CliniMACS: _____

NOTE: Prior to initiation of procedure on CliniMACS ensure the blue clamp of the transfer set is open/unclamped.

Date/time sample loaded on CliniMACS: _____

Cell Processing – Post Magnetic Separation

Process Code Number: _____

Weigh the cell collection bag (subtract the pre-determined tare weight): _____

Post-Selection Testing (Sample C)

Remove 2.0 mL for cell count, viability, flow, HPCA, endotoxin, and Gram stain: _____

Product Cell Count ($\times 10^6$): _____ Product Volume (mL): _____

Total Cells ($\times 10^9$): _____ Viability: _____

Sample taken to Flow and HPCA: _____

Sample sent for endotoxin testing: _____

Sample sent for STAT Gram stain: _____

BARCODE*

Post-Selection Wash

Wash Media Prepared (10 mL of 25% HSA to 100 mL Plasma-Lyte A): _____

If < 100mL

Transfer ~25 mL of cells to each conical centrifuge tube, fill with wash media: _____

Centrifuge at room temperature at 1800 RPM for 15 minutes, **no brake**: _____**If >100mL**

Volume: _____

Add equal amount of Wash Media (example 30mL of 25% HSA to 300mL Plasma-Lyte A)

Actual amount of Wash Media added: _____ mL.

Total volume of product and Wash Media: _____ mL

Centrifuge at room temperature at 1800 RPM for 15 minutes, **no brake**: _____Remove from centrifuge, let hang 10 minutes, and express **all** supernatant:

- Volume expressed: _____ grams
- Transfer and measure in 60mL syringe, if >60mL transfer to 300mL bag. _____
- Volume of Wash Media to add if volume of cells <50mL: _____
 - Target volume = >50mL)
- Final volume: _____

Mix cells gently and thoroughly: _____

Post-Wash Testing (Sample To Infuse)

Remove 0.2 mL for cell count and viability: _____

Product Cell Count ($\times 10^6$): _____ Product Volume (mL): _____Total Cells ($\times 10^9$): _____ Viability: _____

Sterility Bottles Inoculated (5.0 mL of supernatant/bottle): _____

Post-Wash Percent Recovery: _____

BARCODE*

COMMENTS:

I certify that all reagents and supplies used in the processing of these samples show no signs of contamination, irregularities, defects or flaws.

Date _____ Initials _____

I certify that all heat sealed tubing and all sterile welded tubing used in the processing of these samples exhibit no signs of leakage, irregularities, defects or flaws.

Date _____ Initials _____

I certify that the biological safety cabinet (BSC) used to process these cellular products was cleaned BEFORE and AFTER the procedure.

Date _____ Initials _____

BARCODE*

PROCESSING LOT NUMBERSN/A – Not Applicable (*supply not used*)

* See Supply Management Log

ITEM	SUPPLIER	LOT NUMBER	EXPIRATION DATE	USED
Alcohol Pads	Cardinal Health			
Albumin – human 25%	Duke Pharmacy			
BacT/Alert SA	Biomerieux			
BacT Alert SN	Biomerieux			
ChloraPrep® SEPP Applicators	CareFusion			
Cryogenic Storage Bags (250ml)	Origen			
Cryogenic Storage Bags (50ml)	Origen			
DMSO	Protide Pharm.			
Needles 16 Gauge	BD Medical			
Needles 19 Gauge	BD Medical			
Spinal Needle 18 Gauge	BD Medical			
Plasmalyte-A	Baxter			
Sodium Chloride 0.9%	Hospira			
PBS/EDTA Buffer	CliniMACS			
CliniMACS CD56+ Reagent	CliniMACS			
CliniMACS Tubing Set Ref No. 161-01	CliniMACS			
Plasma Transfer Set (spike-needle)	Fenwal			
Plasma Transfer Set (pinch clamp)	Charter Medical			
Blood Transfusion Filter	Haemonetics			
Sampling Site Coupler	Fenwal			
Sterile Docking Wafers	Terumo Medical			
Stopcock (99% DMSO resistant)	Tyco			
Syringe 3 ml	BD Medical			
Syringe 5 ml	BD Medical			
Syringe 10 ml	BD Medical			
Syringe 20 ml	BD Medical			
Syringe 30 ml	BD Medical			
Syringe 60 ml	BD Medical			
Transfer Pack 300 ml	Fenwal			
Transfer Pack 600 ml	Fenwal			

KEY EQUIPMENT USED IN PROCESSING

EQUIPMENT	MANUFACTURE	SERIAL/ID NUMBER
BSC	BAKER/NUAIRE	
CENTRIFUGE	SORVALL	
CENTRIFUGE	ALLEGRA	
STERILE WELDER	TERUMO	
CONTROL RATE FREEZER	CRYOMED	
CLINIMACS	Miltenyi	
PIPETS	(See Next Page)	

BARCODE*

PIPETS	LOCATION	SERIAL #	Check ✓ if Pipet Used (Leave blank if not used)
Gilson P-20	Processing	G8011587	
Gilson P-20	Processing	M12134J	
Gilson P-20	Processing	N17205D	
Gilson P-20	Processing	C19628D	
Gilson P-20	Processing	K18317A	
Gilson P-20	Processing	K18323A	
Gilson P-200	Processing	E14789J	
Gilson P-200	Processing	M11107G	
Gilson P-200	Processing	A8520517	
Gilson P-200	Processing	D8522323	
Gilson P-200	Processing	N10367D	
Rainin Pipet-Lite XLS L-200	Processing	J1269538T	
Rainin Pipet-Lite XLS L-200	Processing	I1261887T	
Rainin Pipet-Lite XLS L-200	Processing	D1326636T	
Rainin Pipet-Lite XLS L-200	Processing	J1269359T	
Rainin Pipet-Lite XLS L-1000	Processing	C1321620T	
Rainin Pipet-Lite XLS L-1000	Processing	C1321482T	
Rainin Pipet-Lite SL-300	Processing	I0985193K	

TECHNOLOGIST'S SIGNATURE _____

DATE _____

Signature Manifest**Document Number:** STCL-PROC-019 FRM1**Revision:** 04**Title:** CD56+ Selection Worksheet FRM1**Effective Date:** 06 Jul 2021

All dates and times are in Eastern Time.

STCL-PROC-019 FRM1 CD56+ Selection Worksheet FRM1**Author**

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATER002)		22 Jun 2021, 11:51:40 AM	Approved

Management

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATER002)		22 Jun 2021, 11:51:56 AM	Approved

Medical Director

Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		22 Jun 2021, 12:35:24 PM	Approved

Quality

Name/Signature	Title	Date	Meaning/Reason
Richard Bryant (RB232)		24 Jun 2021, 07:11:38 AM	Approved

Document Release

Name/Signature	Title	Date	Meaning/Reason
Sandra Mulligan (MULLI026)		24 Jun 2021, 05:29:40 PM	Approved