



## STEM CELL LABORATORY (STCL)



**DOCUMENT NUMBER:** STCL-PROC-047 FRM1

**DOCUMENT TITLE:**

CliniMACS TCR alpha beta+ T-Cell / CD19+ B-Cell Reduction Worksheet FRM1

**DOCUMENT NOTES:**

### Document Information

**Revision:** 01

**Vault:** STCL-Processing-rel

**Status:** Release

**Document Type:** STCL

### Date Information

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### Control Information

**Author:** WATER002

**Owner:** WATER002

**Previous Number:** None

**Change Number:** STCL-CCR-524

**BARCODE\***

**STCL-PROC-047 FRM1**  
**CliniMACS TCR $\alpha\beta$ + T-Cell / CD19+ B-Cell Reduction Worksheet**

**Recipient Name:** \_\_\_\_\_ **History #:** \_\_\_\_\_ **ABO/Rh:** \_\_\_\_\_

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(\* 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:**

WBC  $40 \times 10^9$ ; 1 vial of CliniMACS Anti-Biotin Reagent Vial; 2 liters CliniMACS PBS/EDTA Buffer

WBC  $80 \times 10^9$ ; 2 vials of CliniMACS Anti-Biotin Reagent Vial; 2 liters CliniMACS PBS/EDTA Buffer

Labeled cells  $< 50 \times 10^8$ ; 1 liter CliniMACS PBD/EDTA Buffer

Label cells  $5-13 \times 10^9$ ; 2 liter CliniMACS PBD/EDTA Buffer; 1000mL Negative fraction bag, Buffer Waster Bag, Cell collection bag

Label cells  $13-20 \times 10^9$ ; 3 liter CliniMACS PBD/EDTA Buffer; 1000mL Negative fraction bag, Buffer Waster Bag, Cell collection bag

**IMPORTANT NOTE:**

Labeling for combined TCR $\alpha\beta$ + / CD19+ depletion is performed within two-step procedure.

**1<sup>st</sup> step:** TCR $\alpha/\beta$  labeling requires a sample volume of 95.0 mL plus one vial of CliniMACS TCR $\alpha\beta$ -Biotin

**2<sup>nd</sup> step:** Anti-Biotin / CD19 labeling requires a sample volume of 175.0 mL plus two vials of CliniMACS Anti-Biotin Reagent plus two vials of CliniMACS CD19 Reagent

**Pre-Overnight Testing (if applicable)**

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

Product Cell Count (x 10<sup>6</sup>): \_\_\_\_\_ Product Volume (mL): \_\_\_\_\_

Total Cells (x 10<sup>9</sup>): \_\_\_\_\_ Viability: \_\_\_\_\_

Sterility Bottles Inoculated: \_\_\_\_\_ Nuncs: \_\_\_\_\_

**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 (x 10<sup>6</sup>): \_\_\_\_\_ Product Volume (mL): \_\_\_\_\_

Total Cells (x 10<sup>9</sup>): \_\_\_\_\_ Viability: \_\_\_\_\_

Sterility Bottles Inoculated: \_\_\_\_\_ Nuncs: \_\_\_\_\_

Sample taken to Flow and HPCA: \_\_\_\_\_

Working Buffer Prepared (20 mL of 25% HSA to 3 liters of PBS/EDTA buffer): \_\_\_\_\_

**Platelet Wash (Dilution Step):**

**Dilute the product 1:3 with 0.5% HSA-supplemented CliniMACS PBS/ADTA Buffer, but not exceeding a total volume 600ml**

Transfer well mixed product to a labeled 600 mL transfer pack and weigh to obtain volume: \_\_\_\_\_grams.

Volume of product multiple by 2: \_\_\_\_\_grams (amount of buffer to add)

Actual amount of PBS added: \_\_\_\_\_grams

Total volume of product and buffer: \_\_\_\_\_grams

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Stem Cell Laboratory, DUMC  
Durham, NC

Centrifuge at room temperature at 980 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 =  $95 \pm 5$  mLs for  $< 60 \times 10^9$  TNC (95grams-106grams)
- Final volume: \_\_\_\_\_ grams (divide by 1.06 to convert to mL) \_\_\_\_\_ mL

Mix cells gently and thoroughly: \_\_\_\_\_

### **Adding IVIG:**

Volume IVIG added: \_\_\_\_\_ mL

Incubate for 5 minutes: \_\_\_\_\_

### **Cell Processing – 1<sup>st</sup> Labeling**

#### **Primary labeling of TCR $\alpha$ / $\beta$ positive cells:**

One vial of CliniMACS TCR $\alpha$  $\beta$ -Biotin Reagent (7.5 mL) is sufficient for **60 x 10<sup>9</sup> TNC** and **24 x 10<sup>9</sup> TCR $\alpha$ / $\beta$ -positive cells**

Optimal labeling requires a sample volume of 95 mL plus the entire volume of one vial of CliniMACS TCR $\alpha$ / $\beta$ -Biotin.

Inject **COLD** TCR $\alpha$  $\beta$ -Biotin (followed by a syringe of air): \_\_\_\_\_

Amount of reagent injected: \_\_\_\_\_

Time reagent injected/incubation start time: \_\_\_\_\_

Incubation stop time: \_\_\_\_\_

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

### **Wash I:**

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 1200 RPM for 15 minutes, **no brake**: \_\_\_\_\_

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

- Volume expressed: \_\_\_\_\_grams

Mix cells gently and thoroughly: \_\_\_\_\_

## **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 1200 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

***\*\* Adjusting the cellular product; transfer cell into new 600ml bag, filter clumped (if needed) \*\****

- Volume of buffer to add: \_\_\_\_\_grams
  - Target volume =  $175 \pm 5$  mLs (186 grams-196 grams)
- Final volume: \_\_\_\_\_grams (divide by 1.06 to convert to mL) \_\_\_\_\_mL

Mix cells gently and thoroughly:

## **Cell Processing – 2<sup>nd</sup> Labeling**

### **Secondary labeling of TCR $\alpha\beta$ -positive cells:**

Two vials of CliniMACS Anti-Biotin Reagent (2x7.5ml) are sufficient for labeling of a cell product containing up to **80 x 10<sup>9</sup> TNC**

Two vials of CliniMACS CD19 Reagent (2x7.5ml) are sufficient for labeling up to **10 x 10<sup>9</sup> CD19-positive cells** out of total up to **80 x 10<sup>9</sup> TNC**

Optimal labeling requires a sample volume of 175ml plus the entire volume of two vials of CliniMACS Anti-Biotin Reagent and two vials of CliniMACS CD19 Reagent

Inject **COLD** Anti-Biotin Reagent (followed by a syringe of air): \_\_\_\_\_

Amount of reagent injected: \_\_\_\_\_

Time reagent injected: \_\_\_\_\_

Inject cold CD19 Reagent: \_\_\_\_\_

Amount of reagent injected: \_\_\_\_\_

Time reagent injected/incubation start time: \_\_\_\_\_

Incubation stop time: \_\_\_\_\_

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

### **Wash III:**

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 =  $150 \pm 5$  mLs (154 grams to 164 grams)
- Final volume: \_\_\_\_\_grams (divide by 1.06 to convert to mL) \_\_\_\_\_mL.

**\*\* Ensure the cell concentration adjusted does not exceed  $400 \times 10^6$  WBC**

### **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 – CliniMACS Depletion**

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 300 mL labeled transfer pack: \_\_\_\_\_

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: \_\_\_\_\_

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 of the pre system filter and firmly insert the spike into the pre system filter: \_\_\_\_\_
- Remove the top cap from pre-system filter and connect to the blunt end of Spike Connect (included with set): \_\_\_\_\_
- **NOTE:** Be extremely careful, refer to STCL-PROC-018 for further connection instructions

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 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 bag of buffer: \_\_\_\_\_

Set up the CliniMACS:

- Separation program used: \_\_\_\_\_
- Tubing Set used: \_\_\_\_\_
- Ref. No. of Tubing Set: \_\_\_\_\_
- Lot number of Tubing Set: \_\_\_\_\_
- Expiration date of Tubing Set: \_\_\_\_\_

**Input of sample parameters (Sample B)**

- WBC concentration: \_\_\_\_\_
- Percentage of labeled cells: \_\_\_\_\_  
(IMPORTANT: Consider all TCR $\alpha$ / $\beta$  and CD19-positive events including unspecific binding e.g to dead cells)
- Sample loading volume: \_\_\_\_\_

**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): \_\_\_\_\_

Weight if the Target Cell Fraction \_\_\_\_\_ grams (divide by 1.06 to convert to mL)

**Post-Selection Testing – Target Cell Fraction (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: \_\_\_\_\_

**Non-Target Cell Fraction (Sample D)**

Weight of Non-Target Cell bag (subtract the pre-determined tare weight): \_\_\_\_\_

Remove 0.5mL for cell count and flow

**Optional: Calculation of the weight of the Wash Fraction (Buffer Waste Bag)**

Weight of Buffer Waste Bag (subtract the pre-determined tare weight): \_\_\_\_\_

Remove 10 mL for cell count and flow labeled **wash fraction**

**Post-Selection Wash**

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



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

- **NOTE:** Number of conical centrifuge tubes is determined by product volume.

Centrifuge at room temperature at 1800 RPM for 15 minutes, **no brake**: \_\_\_\_\_

### **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: \_\_\_\_\_

### **Summarizes data analysis:**

<b>Fraction</b>	<b>Volume (mL)</b>	<b>WBC Concentration (<math>\times 10^6/\text{mL}</math>)</b>	<b>Total WBC (<math>\times 10^9</math>)</b>	<b>TCR<math>\alpha</math>/<math>\beta</math> (%)</b>	<b>Total TCR<math>\alpha</math>/<math>\beta</math><sup>+</sup> cells (<math>\times 10^9</math>)</b>	<b>CD19<sup>+</sup> (%)</b>	<b>Total CD19<sup>+</sup> cells (<math>\times 10^9</math>)</b>	<b>Viability (%)</b>
Leukapheresis Product ( <b>Sample A</b> )								
Original (after sample preparation) ( <b>Sample B</b> )								
Target cells = TCR $\alpha$ / $\beta$ /CD19-depleted (Cell collection Bag) ( <b>Sample C</b> )								
Non-Target cells = labeled (Non target cell Bag) ( <b>Sample D</b> )								
Optional: Buffer Waste Bag ( <b>Wash Fraction</b> )								

**Calculation:**

The depletion efficiency (-logP) describes the level of depletion of labeled cells.

$$-\log P = -\log \left( \frac{\# \text{TCR}\alpha\beta^+ \text{ and CD19}^+ \text{ respectively target fraction}}{\# \text{TCR}\alpha\beta^+ \text{ and CD19}^+ \text{ respectively original fraction}} \right)$$

Depletion efficiency of TCR $\alpha\beta^+$  cells: \_\_\_\_\_

Depletion efficiency of CD19 $^+$  cells: \_\_\_\_\_

Another important parameter is the yield concerning how many TCR $\alpha/\beta^-$  and CD19 $^-$  negative cells were lost during the separation procedure

$$\% \text{ yield} = \# (\text{TCR}\alpha\beta^- \text{ and CD19}^-) \text{ target fraction} * 100 / \# (\text{TCR}\alpha\beta^- \text{ and CD19}^-) \text{ origin fraction}$$

Yield TCR $\alpha\beta^-$  and CD19 $^-$  cells: \_\_\_\_\_

**COMMENTS:**


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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 \_\_\_\_\_

**PROCESSING LOT NUMBERS**N/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 AST	Biomerieux			
BacT Alert NST	Biomerieux			
ChloroPrep® 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 TCR $\alpha$ / $\beta$ -Biotin Reagent	CliniMACS			
CliniMACS Anti-Biotin Reagent	CliniMACS			
CliniMACS CD19 Reagent	CliniMACS			
IgG ( <i>Duke Pharmacy</i> )				
CliniMACS Depletion Tubing Set	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			
Filter	Codan			
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			
Stopcock	Smiths Medical			

**KEY EQUIPMENT USED IN PROCESSING**

EQUIPMENT	MANUFACTURE	SERIAL / ID NUMBER	✓ if used or N/A
BSC <i>(Circle ONE)</i>	BAKER NUAIRE OTHER		
CENTRIFUGE # <i>(Complete below if used)</i> <i>Check Centrifuge used (if applicable)</i>	SORVALL RC3B PLUS ALLEBRA	<i>(Check one)</i> <input type="checkbox"/> 100100184 <input type="checkbox"/> AK03F06	
TUBE SEALER	SEBRA	<i>(Check one)</i> <input type="checkbox"/> 0823 <input type="checkbox"/> 0363	
CLINIMACS	MILTENYI	<i>(Check one)</i> <input type="checkbox"/> 408 <input type="checkbox"/> 411	
STERILE WELDER	TERUMO	60071	
CONTROL RATE FREEZER (Model 7452)	CRYOMED ThermoScientific	<i>(Check one)</i> <input type="checkbox"/> 507471-154 <input type="checkbox"/> 507471-155	
MICROSCOPE	OLYMPUS BH-2	216086	
SCALE / BALANCE	METTLER TOLEDO (PG3001) OHAUS (SPX6201)	<i>(Check one)</i> <input type="checkbox"/> 1116372248 <input type="checkbox"/> C039175306	
REFRIGERATOR (Model GP12W1AFR)	REFRIG (COMBO)	0809466-A08129-02	
REFRIGERATOR (Blood Bank)	HARRIS	V26T136614WT	
PIPETS	<i>(Document on next page)</i>		

PIPETS	LOCATION	SERIAL #	Check ✓ if Pipet Used ( <i>Leave blank if not used</i> )
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-047 FRM1**Revision:** 01**Title:** CliniMACS TCR alpha beta+ T-Cell / CD19+ B-Cell Reduction Worksheet FRM1**Effective Date:** 01 Sep 2021

All dates and times are in Eastern Time.

**STCL-PROC-047 FRM1 CliniMACS TCR alpha beta+ T-Cell / CD19+ B-Cell Reduction Worksheet FRM1****Author**

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**Management**

Name/Signature	Title	Date	Meaning/Reason
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**Quality**

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**Document Release**

Name/Signature	Title	Date	Meaning/Reason
Sandra Mulligan (MULLI026)		30 Aug 2021, 08:21:18 PM	Approved