



STEM CELL LABORATORY (STCL)



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DOCUMENT TITLE:

CD45+RA Depletion Worksheet

DOCUMENT NOTES:

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Control Information

Author: WATE02

Owner: WATE02

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**STCL-PROC-038 FRM1
CD45RA+ DEPLETION WORKSHEET**

Barcode

Recipient Name: _____ HX#: _____ ABO/Rh: _____

Donor Name: _____ HX#: _____ ABO/Rh: _____

Recipients weight: _____ Date of Procedure: _____

Performing Technologist: _____

Pre Sample Testing (Sample A)

Cell Count: _____ Volume: _____ Total Cells: _____ HPCA: _____

Viability: _____ %CD45RA+ cells: _____ Total # CD45RA+ cells: _____

Centrifuge at room temperature: _____

Working buffer prepared: _____

Target volume of 200 mls and cell count $\leq 200 \times 10^6/\text{ml}$ achieved: _____

Wash the Cells

Label a 600 ml transfer pack "cell preparation" and weigh bag and tare scale _____

Transfer well mixed product to this bag and weigh to obtain volume _____

Fill the bag with buffer _____

Centrifuge at RT 200G (800 RPM Sorvall or 1020 RPM Allegra) for 15 minutes, no brake

Express supernatant and if necessary adjust volume to 95mls +/- 5mls with buffer _____

Mix cells gently and thoroughly _____

Cell Processing - Labeling

Inject one vial cold CD45RA reagent _____

Inject several syringes of air and mix well _____

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

(NOTE: Capacity of CD45RA reagent and DTS is 20×10^9 CD45+ cells out of a TNC $\leq 50 \times 10^9$)

Wash the Cells

Fill the bag with buffer _____

Centrifuge at RT 300G (1000 RPM Sorvall or 1250 RPM Allegra) for 15 minutes, no brake _____

Express supernatant and mix cells gently and thoroughly _____

Pre Depletion Testing (Sample B)

Adjust volume to 125 mls with buffer and mix gently and thoroughly _____

Cell Count: _____ Volume: _____ Total Cells: _____ HPCA: _____

Viability: _____ %CD45RA+ cells: _____ Total # CD45RA+ cells: _____

(NOTE: A maximum cell concentration of 0.4×10^9 cells per mL of labeled/washed cells should not be exceeded for loading on the tubing set.)

Cell Processing – Magnetic Separation

CliniMACS prepared for Depletion 3.1 and tubing set installed _____

Cell Processing – Post Magnetic Separation

Record Process Code _____

Weigh the cell collection bag (subtract tare weight of 32 g) _____

Post Depletion Testing (Sample C)

Cell Count: _____ Volume: _____ Total Cells: _____

Viability: _____ %CD45RA+ cells: _____ Total # CD45RA+ cells: _____

Gram Stain: _____ HPCA: _____

Non-Targeted Cell Bag (NTCB) Volume _____ Cell Count: _____

Endotoxin and Sterility testing performed on Non-Target Cell Bag _____

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Stem Cell Laboratory, DUMC

Durham, NC

Comments: _____

Reagents and Supplies

ITEM	SUPPLIER	LOT NUMBER	EXP. DATE	USED
25% HSA				
Alcohol Prep Pads				
BacT/Alert SA	bioMerieux			
BacT/Alert SN	bioMerieux			
Buffer	CliniMACS			
CD45RA reagent	CliniMACS			
ChloraPrep®SEPP Applicators	Cardinal Health			
Filter	Pall			
Needles 16 gauge	BD			
Needles 19 gauge	BD			
Plasma Transfer Set (<i>spike/spike</i>)	Fenwal			
Sampling Site Coupler	Fenwal			
Sterile Docking Wafer	Terumo			
Syringe 3 ml	BD			
Syringe 10 ml	BD			
Syringe 20 ml	BD			
Syringe 30 ml	BD			
Syringe 60 ml	BD			
Transfer Pack 150 ml	Baxter/Fenwal			
Transfer Pack 300 ml	Fenwal			
Transfer Pack 600 ml	Fenwal			
Tubing Set	CliniMACS			

Equipment:**Serial #**

BSC	
Centrifuge	
CliniMACS	
Sterile Welder	

TECHNOLOGIST'S SIGNATURE _____ **DATE** _____

I certify that all reagents and supplies used in processing these samples show no signs of contamination, irregularities, defects or flaws. Also, all heat sealed tubing and all sterile dockings exhibited no signs of leakage, irregularities, defects or flaws.

Date: _____

Initials: _____

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Signature Manifest

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All dates and times are in Eastern Time.

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