STEM CELL LABORATORY (STCL)

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DOCUMENT TITLE:

Miltenyi Large Scale CCB \(IFN-gamma\) Worksheet FRM1

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Barcode

STCL-PROC-037 FRM1
MILTENYI LARGE SCALE CCS (IFN-gamma) WORKSHEET

Donor Name: _____ History No.:_____
Recipient Name: _____ History No.:_____
Collection Date and time: _____ Bag labels checked by: _____
Selection Date: _____ Performing technologist: _____

Pre Selection

Centrifuge turned off by: _____ Time: _____ RPMI brought to room temperature by: _____
AB Serum thawed by: _____ AB Serum filtered by: _____
RPMI with 2% by volume filtered AB serum prepared by: _____
mls RPMI: _____ mls AB serum: _____ Transferred to 600ml transfer pack placed in refrigerator by: _____
(600mls will be needed)
RPMI with 10% by volume filtered AB serum prepared by: _____
mls RPMI: _____ mls AB serum: _____ Transferred to 600ml transfer packs and placed in 37°incubator by: _____
(1200mls will be needed)
R.T. RPMI transferred to 1000 ml bag: ____ Transferred by: _____
(Transfer by aseptically pouring RPMI through 60cc syringe attached to luer adapter)
3 liters of working buffer prepared, labeled and placed in the refrigerator by: _____
300ml transfer pack prepared and weighed for final cell collection by: _____
600ml transfer packs labeled appropriately by: _____
Hood inspected, cleaned and disinfected prior to initiating procedure by: _____

Initial Product (Sample A)

Cell count: _____ Volume: _____ Total Cells: _____ Viability: _____
Product Cultured: _____ Flow sent _____ HPCA sent_____ ABO: _____ by ____
Calculations for $1x10^9$ cells:

Cell Processing - Washing

Inject $1x10^9$ cells into 600 ml transfer pack through a luer adapter and fill bag to 500cc with R.T. RPMI. Centrifuge at R.T. at 200xg for 10 minutes with no brake. Express supernatant.
Perform a second wash of the $1x10^9$ cells in the same manner.
First wash completed by: _____ Second wash completed by: _____
Post 2nd wash, supernatant expressed to minimum volume by _____ Volume = _____
(target=40mls)
Volume of 37°C RPMI with 10% AB serum to add to cell prep:
(100mls – volume remaining in bag) = _____ Calculated and added by_____

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Cell Processing – Re-stimulation

Transfer to a gas-permeable cell culture bag and add 1 ml of pp65. Added by _____

Incubate for 4 hours at 37°C. Time incubation started: _____ Timer set: _____
Note: Target is 100ml of the product with a cell density of 0.5 to 1.0X10^7 cells/cm^2 and 1x10^7 cells/mL.

During Incubation

Fill three 600 ml transfer packs with 125 mls warm RPMI with 10%AB serum, label as #1, #2 and #3 and return to incubator. Filled, labeled and stored by: _____
Verify a chilled (4°C) centrifuge is ready.

Cell Processing – Labeling (IFN-gamma Catchmatrix Reagent)

Product removed from incubator by: _____ Product transferred to a 600ml transfer pack and filled with cold (2-8°C) RPMI with 2% AB serum added by: ___
Product centrifuged at 4-8°C at 300xg for 10 minutes with no brake by: _____
(During centrifugation, prepare 4 buckets of ice).
Supernatant expressed and cells re-suspended by: _____
Volume of cells remaining: _____ (target is smallest volume feasible)
Catch Matrix injected into cell mixture by: _____ (20g needle/10cc syringe)
Sample gently mixed, placed on ice and timer set for **5 minutes** by: _____
500ml of 37°C RPMI with 10% AB serum added to product bag by: _____
Timer immediately set for 45 minutes by: _____ at _____
Product mixed thoroughly. 125mls from product bag dispensed into each 600ml bag containing warm media (in order of 1,2,3) and 125ml warm media dispensed into remaining product bag. The result is 4 bags containing 250mls each. Bags filled by: _____
Bags placed in incubator for remainder of the 45 minutes, mixing bags gently every 5 minutes.

Cell Processing – Post Labeling

Product removed from incubator by: _____ at _____.
250mls cold working buffer added to each bag by: _____ Each bag placed on ice for 10 minutes by: _____ (timer set)
Bags centrifuged at 300xg with no brake for 10 minutes at 4°C by: _____
Supernatant in each bag expressed by: _____(approximately 500ml total)
200mls cold working buffer added to bag#1 by: _____. All 4 bags thoroughly mixed, rinsed and recombined into one bag by: _____
Cells centrifuged at 300xg without brake for 10 minutes at 4°C by: _____

Cell Processing – Magnetic Labeling

Supernatant expressed and cells re-suspended by: _____
Volume of cells remaining: _____ (target is smallest volume feasible)
Microbeads injected into cell mixture by: _____ (20g needle/10cc syringe)
Sample gently mixed, placed on ice and timer set for 15 minutes by: _____
After the 15 minutes incubation, bag filled with cold working buffer by: _____
Sample centrifuged at 300xg without brake for 10 minutes at 4-8°C by: _____
Supernatant removed by: _____ Volume adjusted to 100mls with cold working buffer by: _____ Sample removed for analysis by: _____

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Pre Selection Product (Sample B)
Cell count: _____ Volume: _____ Total Cells: _____ Cell Recovery: _____
Flow sent _____ HPCA sent_____ Viability: _____

Cell Processing – Magnetic Separation
Sample collection bag prepared and weighed by: _____ Tare weight of bag: _____
CliniMACS prepared for Enrichment 3.2 and tubing set installed by: _____

Cell Processing – Post Magnetic Separation
Weight of end product: _____ minus tare weight of cell collection bag = volume of final product: _____
Product concentrated and reconstituted to _____ mls by _____.

Post Selection Product (Sample C – Pre-Concentration)
Cell count: _____ Volume: _____ Total Cells: _____ Flow sent on product_____, negative fraction _____ and buffer waste bag _____ HPCA sent_____ Product viability: _____

Post Selection Product, Post Concentration (Sample C – Post-Concentration)
Cell count: _____ Volume: _____ Total Cells: _____ Culture (21CFR.610.12) bacterial _____ fungal _____ gram stain _____ (MICRO LAB); culture_____ (STCL), Endotoxin _____ Flow sent on product bag _____

Supplies and Reagents

ITEM	SUPPLIER	LOT NUMBER	EXPIRATION DATE	USED
Alcohol Pads				
BacT/Alert SA				
BacT/Alert SN				
Plasma Transfer Sets (spike/spike)				
Needles (16 gauge)				
Needles (20 gauge)				
Sampling Site Coupler				
Sterile Docking Wafers				
Syringe (1ml)				
Syringe (5ml)				
Syringe (10ml)				
Syringe (20ml)				
Syringe (30ml)				
Syringe (60ml)				
Transfer Pack (150ml)				
Transfer Pack (300ml)				
Transfer Pack (600ml)				

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ITEM	SUPPLIER	LOT NUMBER	EXPIRATION DATE	USED
RPMI				
AB Serum				
25% HAS				
Gas Permeable Culture Bag				
CytoStim				
Anti-IFNj-PE, human				
Buffer				
Tubing Set				
CMV pp65				
CCS (IFN-gamma)Microbeads				
Pall Filter				
Blood Filter				
Sterile Caps				
Plasma Transfer Set				
Luer/Spike Interconnectors				
24 Well Culture Dish				
Pre-Separation Filters				
15ml Sterile Conicals				
50ml Sterile Conicals				

Equipment

	Make	Model	Serial Number
CliniMACS			
Sterile Docker			
MiniMACS			
MACSmix			
Centrifuge			
Centrifuge			
Balance			
Tubing Sealer			
BSC			
Cell Counter			

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

Date _____ Initials _____

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

Date _____ Initials _____

Signature Manifest

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STCL-PROC-037 FRM1 Miltenyi Large Scale CCB (IFN-gamma) Worksheet

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