

Appendix A: Cross-Network PBMC Processing Worksheet v7.0

Note: The fields in this worksheet must be filled out by hand, using a pen.

Specimen Processing Laboratory:		Protocol:		
Participant ID (PTID/PID):		Visit Number:	Visit Type:	
Collection Date:		Collection Time:		
Processing Start Date:		Processing Start Time:	Processed By (Initials):	
Reagents	Manufacturer	Lot Number		Expiration Date
DMSO				
FBS				
WDR: HBSS or PBS (circle one)				
Cell Separation Tube (frit)				
Density Gradient Media				
	Volume in mL (record as X.Y)			Expiration Date
CPS	CPS	DMSO	FBS	1 working day (<18hrs)
Data to be Captured During Processing				Sample
Sample tube type (circle one or record "other" tube type)				ACD / HEP / EDT Other: _____
Blood condition (circle one or more; add comments on reverse as needed)				SAT/ HEM / CLT
Measured usable whole blood volume (to the nearest 0.1mL)				mL
Indicate processing method (circle one)				CSTFB / overlay / underlay
Counting Method: Name of specific instrument or manual count (record in field to right)				
Counting re-suspension volume of HBSS (or other WDR) (V) (record as X.Y)				mL
Cell count average concentration (C)				x 10 ⁶ cells/mL
Total cell number (T) = C x V				x 10 ⁶ cells
Calculate cell yield/mL of whole blood (QC check)= (T/Usable Whole Blood Volume)				x 10 ⁶ cells/mL
Calculate estimated CPS re-suspension vol. (V1)=(T/15x10⁶ cells/mL)(1mL)				mL
Calculate final CPS re-suspension volume (Vf), (V1 rounded DOWN to nearest whole (X.0) mL)				mL
Calculate actual number of cells per vial N2 = (T/ Vf) x V2; (V2=1 mL).				x 10 ⁶ cells/vial
Print and QC LDMS Label content/barcodes (initials of person (s) performing QC)				
Frozen Date and Time (ddMMMyyyy /HH:MM) (Explain in comments section if not within 4 hours of processing start time)				
Number of Cryovials actually frozen Note: Should be equal to final CPS re-suspension volume for 1mL aliquots (Vf).				
Complete remaining LDMS entries including total cell count & frozen time (Initials)				

Appendix A: Cross-Network PBMC Processing Worksheet v7.0 (Page 2 of 2)

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Specimen Processing Laboratory:

PTID/PID:

Transfer of Cryovials to Freezer Storage Box	
Person who transferred cryovials to storage box locations assigned by LDMS	
Date (ddMMMyyyy)/time cryovials were transferred from controlled-rate freezing device to storage box. (Sample must be maintained at -80°C during transfer)	
Initial (Primary) Review (Initials/Date)	
Final (Secondary) Review (Initials/Date)	

Hemocytometer Counts	Total Count	Viable Cells	Non-Viable
Square #1 (cells/mm ²)			
Square #2 (cells/mm ²)			
Square #3 (cells/mm ²)			
Square #4 (cells/mm ²)			
Average Cell Count per Square (cells/mm ²)			
PBMC Dilution Factor (1:DF*)			
Hemocytometer Factor for cells/mL	10 ⁴	10 ⁴	10 ⁴
Cell count concentration (C) = (Average Cells/mm ²)(DF)(10 ⁴); convert to 10 ⁶ cells/mL	Not applicable	x 10 ⁶ cells/mL	Not applicable
% viability = (Viable cells 4 squares/total cells 4 squares) (100)	Not applicable		Not applicable

*Note: Dilution Factor (DF) = (parts cells + parts dilution fluid)/ parts cells

Automated Cell Counts (10 ³ /μl=10 ⁶ /mL)	Count #1
Cell Count (C) as cells x 10 ⁶ /mL	
PBMC Dilution Factor (1:DF**)	
Cell Concentration = (C)(DF)	x 10 ⁶ cells/mL
% viability (if applicable)	

**Note: Dilutions for automated counters are extremely rare. If performing direct counts, enter a 1 in the DF box and complete the column.

Comments, protocol deviations, and additional information not captured elsewhere in this worksheet: