Appendix A: Cross-Network PBMC Processing Worksheet v7.0 (Page 1 of 2)

Note: The fields in this worksheet must be filled out by hand, using a pen. Refer to Protocol LPC (for IQA certification/testing then four aliquots of 3-5M cells)

Specimen Processing Laboratory: | Submission type (circle one): IQA or Protocol

Protocol Number (N/A if IQA): | N1: | A*:

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	DMSO	FBS	
CPS				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 vol. **(WBV)** (to the nearest 0.1mL)	mL
Measured plasma vol. removed and replaced with equal volume of WDR (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 vol. of WDR **(V) = WBV x 0.20** (round DOWN to nearest whole (X.0) mL)	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
If ***T/A ≥ N1***; then CPS re-suspension vol ***(V1) = A*** If ***T/A < N1***; then calculate estimated CPS re-suspension vol. ***(V1)=(T/N1x10^6 cells/mL)(1mL)***	mL
Calculate final CPS re-suspension vol. (V_f), (V1 rounded DOWN to the nearest whole (X.0)mL)	mL
Calculate actual number of cells per vial. ***N2*** = ***(T/V_f)*** **x V2**; (V2=1 mL) ***Note:*** Do not store more than 50M cells per vial	x 10^6 cells/vial
Number of Cryovials actually frozen ***Note:*** Should be equal to final CPS re-suspension volume for 1mL aliquots (V_f) and **≤ (A)**	
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)	
Complete remaining LDMS entries including total cell count & frozen time (Initials)	

Note:* **A = The maximum number of aliquots required according to the protocol-specific Laboratory Processing Chart (LPC). Do not store more than this number of aliquots.

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

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

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)	

Hemacytometer 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**)			
Hemacytometer 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 10^6cells/mL	Not applicable
% viability = (Viable cells 4 squares/total cells 4 squares) (100)	Not applicable		Not applicable

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 10^6cells/mL
% viability (if applicable)	

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

*****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:

Note: **(A) =** The maximum number of aliquots required according to the protocol-specific Laboratory Processing Chart (LPC). Do not store more than this number of aliquots.

Cross-Network PBMC Processing Standard Operating Procedure

Appendix A: Cross-Network PBMC Processing Worksheet v7.0 ***Ex: N1 = 20 mil cells; A = 5 aliquots***

Note: The fields in this worksheet must be filled out by hand, using a pen. Refer to Protocol LPC (for IQA certification/testing then four aliquots of 3-5M cells)

Specimen Processing Laboratory: Lab 398	Submission type (circle one): IQA or (Protocol)	
Protocol Number (N/A if IQA): 313	N1: 20 mil cells	A*: 5 aliquots
Participant ID (PTID/PID): 123-456789	Visit Number: 2.0	Visit Type: vst
Collection Date: 08AUG2024	Collection Time: 08:00	
Processing Start Date: 08AUG2024	Processing Start Time: 08:45	Processed By (Initials): CN

Reagents	Manufacturer	Lot Number	Expiration Date
DMSO	Sigma	RNBM0548	18JAN2025
FBS	Peak	13G1212	18AUG2025
WDR: HBSS or PBS (circle one)	Gibco	2660057	30APR2026
Cell Separation Tube (frit)	Greiner	E220337Q	14MAR2027
Density Gradient Media	Cytiva	1Q345061	31AUG2026

	Volume in mL (record as X.Y)			Expiration Date
	CPS	DMSO	FBS	
CPS Prepared 19AUG2024 08:30 CN	9.0	0.9	8.1	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 vol. **(WBV)** (to the nearest 0.1mL)	86.3 mL
Measured plasma vol. removed and replaced with equal volume of WDR (to the nearest 0.1mL)	40.2 mL
Indicate processing method (circle one)	(CSTFB) / overlay / underlay
Counting Method: Name of specific instrument or manual count (record in field to right)	Manual Count
Counting re-suspension vol. of WDR **(V) = WBV x 0.20** (round DOWN to nearest whole (X.0) mL)	17.0 mL
Cell count average concentration **(C)**	7.2 x 10^6 cells/mL
Total cell number **(T) = C x V**	122.4 x 10^6 cells
Calculate cell yield/mL of whole blood. (QC check)= (T/Usable Whole Blood Volume)	1.4 x 10^6 cells/mL
If ***T/A ≥ N1***; then CPS re-suspension vol ***(V1) = A*** If ***T/A < N1***; then calculate estimated CPS re-suspension vol. ***(V1)=(T/N1x10^6 cells/mL)(1mL)***	5.0 mL
Calculate final CPS re-suspension vol. (V_f), (V1 rounded DOWN to the nearest whole (X.0)mL)	5.0 mL
Calculate actual number of cells per vial. ***N2*** = ***(T/V_f)*** **x V2**; (V2=1 mL) ***Note:*** Do not store more than 50M cells per vial	24.4 x 10^6 cells/vial
Number of Cryovials actually frozen ***Note:*** Should be equal to final CPS re-suspension volume for 1mL aliquots (V_f) and **≤ (A)**	5
Print and QC LDMS Label content/barcodes (initials of person (s) performing QC)	CN
Frozen Date and Time (ddMMMyyyy /HH:MM) (Explain in comments section if not within **4 hours** of processing start time)	08AUG2024 / 10:30
Complete remaining LDMS entries including total cell count & frozen time (Initials)	CN

Note:* **A = The maximum number of aliquots required according to the protocol-specific Laboratory Processing Chart (LPC). Do not store more than this number of aliquots.

Example:
N1 = 20x10^6 cells/mL
A = 5 aliquots

Calculations:

CPS re-suspension volume (V1)

122.4/5 = 24.4>20

Thus, T/A ≥ N1

(V1) = A

Actual number of cells per vial (N2)

122.4/5 x 1 = 24.4x10^6 cells/vial

Note: **(A) =** The maximum number of aliquots required according to the protocol-specific Laboratory Processing Chart (LPC). Do not store more than this number of aliquots.

Cross-Network PBMC Processing Standard Operating Procedure

Appendix A: Cross-Network PBMC Processing Worksheet v7.0 ***Ex: N1 = 10 mil cells; A = 5 aliquots***

Note: The fields in this worksheet must be filled out by hand, using a pen. Refer to Protocol LPC (for IQA certification/testing then four aliquots of 3-5M cells)

Specimen Processing Laboratory: Lab 398	Submission type (circle one): IQA or Protocol	
Protocol Number (N/A if IQA): 313	N1: 10 mil cells	A*: 5 aliquots
Participant ID (PTID/PID): 123-456789	Visit Number: 2.0	Visit Type: vst
Collection Date: 08AUG2024	Collection Time: 08:00	
Processing Start Date: 08AUG2024	Processing Start Time: 08:45	Processed By (Initials): CN

Reagents	Manufacturer	Lot Number	Expiration Date
DMSO	Sigma	RNBM0548	18JAN2025
FBS	Peak	13G1212	18AUG2025
WDR: HBSS or PBS (circle one)	Gibco	2660057	30APR2026
Cell Separation Tube (frit)	Greiner	E220337Q	14MAR2027
Density Gradient Media	Cytiva	1Q345061	31AUG2026

	Volume in mL (record as X.Y)			Expiration Date
	CPS	DMSO	FBS	
CPS Prepared 19AUG2024 08:30 CN	9.0	0.9	8.1	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 vol. **(WBV)** (to the nearest 0.1mL)	46.3 mL
Measured plasma vol. removed and replaced with equal volume of WDR (to the nearest 0.1mL)	20.2 mL
Indicate processing method (circle one)	(CSTFB) / overlay / underlay
Counting Method: Name of specific instrument or manual count (record in field to right)	Manual Count
Counting re-suspension vol. of WDR **(V) = WBV x 0.20** (round DOWN to nearest whole (X.0) mL)	9.0 mL
Cell count average concentration **(C)**	4.2 x 10^6 cells/mL
Total cell number **(T) = C x V**	37.8 x 10^6 cells
Calculate cell yield/mL of whole blood. (QC check)= (T/Usable Whole Blood Volume)	0.8 x 10^6 cells/mL
If ***T/A ≥ N1***; then CPS re-suspension vol ***(V1) = A*** If ***T/A < N1***; then calculate estimated CPS re-suspension vol. ***(V1)=(T/N1x10^6 cells/mL)(1mL)***	3.7 mL
Calculate final CPS re-suspension vol. (V_f), (V1 rounded DOWN to the nearest whole (X.0)mL)	3.0 mL
Calculate actual number of cells per vial. ***N2*** = ***(T/V_f)*** **x V2**; (V2=1 mL) ***Note:*** Do not store more than 50M cells per vial	12.6 x 10^6 cells/vial
Number of Cryovials actually frozen ***Note:*** Should be equal to final CPS re-suspension volume for 1mL aliquots (V_f) and **≤ (A)**	3
Print and QC LDMS Label content/barcodes (initials of person (s) performing QC)	CN
Frozen Date and Time (ddMMMyyyy /HH:MM) (Explain in comments section if not within **4 hours** of processing start time)	08AUG2024 / 10:30
Complete remaining LDMS entries including total cell count & frozen time (Initials)	CN

Note:* **A = The maximum number of aliquots required according to the protocol-specific Laboratory Processing Chart (LPC). Do not store more than this number of aliquots.

Example:
N1 = 10x10^6 cells/mL
A = 5 aliquots

Calculations:

CPS re-suspension volume (V1)

37.8/5 = 7.56<10

Thus, T/A < N1

(V1) = 37.8/10x10^6 cells/mL)(1mL)

Actual number of cells per vial (N2)

37.8/3 = 12.6x10^6 cells/vial