

Appendix B: ACTG PBMC Processing Worksheet v8.0 (Page 1 of 2)

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

Refer to Protocol LPC for N1 and A (for IQA certification/testing refer to current program description).

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	Visual QC (yes/no)	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	1 working day (<18hrs)
			FBS	
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 Other: _____
Measured usable whole blood volume (WBV) (to the nearest 0.1mL)				mL
Measured plasma volume removed & 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 volume of WDR (V) = WBV x 0.20 (rounded to the 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 $T/A \geq N1$; then CPS re-suspension vol (V1) = A* If $T/A < N1$; then calculate estimated CPS re-suspension vol. (V1)=($T/N1 \times 10^6 \text{ cells}$)(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/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 (Vf) and $\leq$ (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. For IMPAACT please follow protocol defined requirements.

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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)	

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:** 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.

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

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

ACTG/IMPAACT PBMC Processing Worksheet

Example #1

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Note: The fields in this worksheet must be filled out by hand, using a pen. **Ex: N1 = 20 mil cells; A = 5 aliquots**
Refer to Protocol LPC for N1 and A (for IQA certification/testing refer to current program description).

Specimen Processing Laboratory: Lab #2345		Submission type (circle one): IQA or Protocol		
Protocol Number (N/A if IQA): ACTG 2345		N1: 20 mil cells		A*: 5 aliquots
Participant ID (PTID/PID): 234-567890		Visit Number: 2.0		Visit Type: vst
Collection Date: 01SEP2026		Collection Time: 08:00		
Processing Start Date: 01SEP2026		Processing Start Time: 08:45		Processed By (Initials): AB

Reagents	Visual QC (yes/no)	Manufacturer	Lot Number	Expiration Date
DMSO	Yes	Sigma	041K2318	01DEC2026
FBS	Yes	Sigma	22L538	30NOV2027
WDR: HBSS or PBS (circle one)	Yes	Gibco	2660057	30MAY2027
Cell Separation Tube (frit)	Yes	Sigma	A2055-10EA	14MAR2027
Density Gradient Media	Yes	Cytiva	1Q345061	31AUG2028

CPS Prepared 01SEP2026 08:00 AB	Volume in mL (record as X.Y)			Expiration Date
	CPS	DMSO	FBS	
	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 Other: _____
Measured usable whole blood volume (WBV) (to the nearest 0.1mL)	86.3 mL
Measured plasma volume removed & 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 volume of WDR (V) = WBV X 0.20 (rounded to the 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	122.2 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/N1x10⁶ cells)/(1mL)	5.0 mL
Calculate final CPS re-suspension volume (Vf), (V1 rounded DOWN to nearest whole (X.0) mL)	5.0 mL
Calculate actual number of cells per vial. N2 = (T/Vf) 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 (Vf) and ≤ (A)*	5
Print and QC LDMS Label content/barcodes (initials of person (s) performing QC)	AB
Frozen Date and Time (ddMMMyyyy /HH:MM) (Explain in comments section if not within 4 hours of processing start time)	01SEP2026 / 10:30
Complete remaining LDMS entries including total cell count & frozen time (Initials)	AB

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. For IMPAACT please follow protocol defined requirements.

Example:

N1 = 20x10⁶
cells/mL
A = 5 aliquots

Calculations:

Counting re-suspension volume (V) = WBV x 0.20

(V) = 86.3 x 0.20
(V) = 17.26, rounded to nearest whole mL
(V) = 17.0

CPS re-suspension volume (V1)

122.2/5 = 24.4 > 20

Thus, **T/A ≥ N1**; and
(V1) = A

(V1) = 5

Actual number of cells per vial (N2) = (T/Vf)

(N2) = 122.2/5 x 1
(N2) = 24.4 x 10⁶
cells/vial

ACTG/IMPAACT PBMC Processing Worksheet Example #2

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Note: The fields in this worksheet must be filled out by hand, using a pen. Ex: N1 = 20 mil cells; A = 5 aliquots
Refer to Protocol LPC for N1 and A (for IQA certification/testing refer to current program description).

Specimen Processing Laboratory: Lab #2345		Submission type (circle one): IQA or <u>Protocol</u>		
Protocol Number (N/A if IQA): ACTG 2345		N1: 20 mil cells		A*: 5 aliquots
Participant ID (PTID/PID): 234-567890		Visit Number: 2.0		Visit Type: <u>vs</u>
Collection Date: 01SEP2026		Collection Time: 08:00		
Processing Start Date: 01SEP2026		Processing Start Time: 08:45		Processed By (Initials): AB
Reagents	Visual QC (yes/no)	Manufacturer	Lot Number	Expiration Date
DMSO	Yes	Sigma	041K2318	01DEC2026
FBS	Yes	Sigma	22L538	30NOV2027
WDR: HBSS or <u>PBS</u> (circle one)	Yes	Gibco	2660057	30MAY2027
Cell Separation Tube (frit)	Yes	Sigma	A2055-10EA	14MAR2027
Density Gradient Media	Yes	Cytiva	1Q345061	31AUG2028
		Volume in mL (record as X.Y)		Expiration Date
CPS Prepared 01SEP2026 08:00 AB		CPS	DMSO	FBS
		9.0	0.9	8.1
Data to be Captured During Processing				Sample
Sample tube type (circle one or record "other" tube type)				ACD / HEP <u>EDT</u> Other: _____
Blood condition (circle one or more; add comments on reverse as needed)				<u>SAT</u> / <u>HEM</u> / CLT Other: _____
Measured usable whole blood volume (WBV) (to the nearest 0.1mL)				86.3 mL
Measured plasma volume removed & replaced with equal volume of WDR (to the nearest 0.1mL)				40.2 mL
Indicate processing method (circle one)				<u>CSTFB</u> / overlay / underlay
Counting Method: Name of specific instrument or manual count (record in field to right)				Manual count
Counting re-suspension volume of WDR (V) = WBV X 0.20 (rounded to the 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				122.2 x 10 ⁶ cells
Calculate cell yield/mL of whole blood. (QC <u>check</u>) = (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/N1 x 10 ⁶ cells) / (1mL)				5.0 mL
Calculate final CPS re-suspension volume (V1), (V1 rounded DOWN to nearest whole (X.0) mL)				5.0 mL
Calculate actual number of cells per vial. N2 = (T/V1) 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 (V1) and ≤ (A)*				5
Print and QC LDMS Label content/barcodes (initials of person (s) performing QC)				AB
Frozen Date and Time (dd/MM/yyyy / HH:MM) (Explain in comments section if not within 4 hours of processing start time)				01SEP2026 / 10:30
Complete remaining LDMS entries including total cell count & frozen time (Initials)				AB

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. For IMPAACT please follow protocol defined requirements.

Example:

N1 = 10x10⁶
cells/mL
A = 5 aliquots

Calculations: Counting re-suspension volume (V) = WBV x 0.20

(V) = 46.3 x 0.20
(V) = 9.26, rounded
to nearest whole mL
(V) = 9.0 mL

CPS re-suspension volume (V1)

37.8/5 = 7.5 < 10

Thus, T/A < N1; and
(V1) = (T/N1 x 10⁶
cells) / (1mL)

(V1) = 37.8/10x10⁶
cells/mL (1mL)
(V1) = 3.7 mL

Actual number of cells per vial (N2) = (T/V1) x (V2)

(N2) = 37.8/3 x 1
(N2) = 12.6x10⁶
cells/vial