Appendix B: ACTG PBMC Processing Worksheet (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	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 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)	
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 ≥ N1***; then CPS re-suspension vol ***(V1) = A**** If ***T/A < N1***; then calculate estimated CPS re-suspension vol. ***(V1)=(T/N1x10^6 cells)(1mL)***	mL
Calculate final CPS re-suspension volume (V_f), (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 (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. For IMPAACT please follow protocol defined requirements.

Appendix B: ACTG PBMC Processing Worksheet v8.0 (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: