BIO-FLARE Study

Quality Control Worksheet

BIO-FLARE

Patient Study ID:

Visit number *(circle)* **:** 0 2 5 8 12 24 Ad-hoc 1 Ad-hoc 2

Received

Date: **Time:** **By:**

Serum and urine separation

Tempus Blood RNA tube

	Serum	Urine
Centrifugation start time:		
No. of aliquots frozen *(max. 6)* **:**		
Vol. of aliquots (ml):	0.5	0.9
Freezing time:		

Blood draw time:

Freezing time:
(3 hours from blood draw)

PBMC preparation

Vol. EDTA blood (ml):

Cell counts *(refer to local counting procedure)*

Cells/ml° = no. cells counted in 1mm² x dilution factor x 10⁴ *° assumes depth of 0.1mm*

Total no. of cells = cells/ml x total volume

Couting method used:

Burker ☐
Neubauer ☐
Other *(state)*

	PBMC*	Monocytes	CD4⁺ T cells
Total cell vol.:			
No. cells counted:			
Diution factor:			
Cells/ml:			
Total cell no.:			

Freezing cells

	PBMC (40% total)*	Monocytes	CD4⁺ T cells
Vol. to give 1x10⁵ cells *(1x10⁵ ÷ cells/ml)* **:**	*(white cap)*	*(amber cap)*	*(blue cap)*
No. of aliquots frozen *(orange caps)* **:**	6	1	1
No. of cells per aliquot *(orange caps)* **:**			
Freezing time:			

Cells for flow cytometry purity check

	PBMC	Monocytes	CD4⁺ T cells
Vol. to give 2x10⁵ cells *(2x10⁵ ÷ cells/ml)* **:**	x2	x1	x1

Monocyte and CD4⁺ T cell isolation

CD14 Microbeads	CD4 Microbeads	LS columns (x2)
Lot no.:	**Lot no.:**	**Lot no.:**
Expiry:	**Expiry:**	**Expiry:**

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Monocyte isolation

Total no. PBMC for cell isolation (60% total*):	
No. PBMC for monocyte isolation:	

(Subtract 4x10^5 cells used for the purity check from total no. PBMC for cell isolation)

Vol. MACS buffer (80µl / 10x10^6 PBMC):	µl
Vol. CD14 MicroBeads (20µl / 10x10^6 PBMC):	µl

CD4$^+$ T cell isolation

Added 160µl MACS Buffer? *(Tick)*	
Added 40µl CD4 MicroBeads? *(Tick)*	

CD4$^+$ T cell lysate

QIAzol Lysis Reagent, Lot no.:	
No.of lysed CD4$^+$ T cells:	
Freezing time:	

Purity check by flow cytometry

Well/Tube	Cell type	Stain	Added 6µl antibody cocktail per well? *(Tick)*	Purity (%)
1	PBMC	Unstained	-	-
2	PBMC	Stained		-
3	Monocytes	Stained		
4	CD4$^+$ T cells	Stained		

Antibodies to add to stained well:

Antibody	Vol. per test (µl)	Vol. for 4x cocktail (µl)	Lot no.
CD3 BV421	1µl	4µl	
CD56 FITC	1µl	4µl	
CD8 PE	1µl	4µl	
CD4 PE-Cy7	1µl	4µl	
CD19 APC	0.5µl	2µl	
CD14 APC-Cy7	1µl	4µl	

Notes:

Processed by:	
Signature:	
Date:	