

January 25th, 2023

PMA Batches

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
NDOOG	Adult		?	01/27/21		2	5.29	7.68	20.68		
NDOOG	Adult	20.5 μ l	?	01/27/21		2	5.05	7.02	4.136 \rightarrow 721 μ l		

9:23 AM Thaw

9:51 AM Count (no stain)

10:20 AM Incubation start

(w/ 6 μ l CD107A)

4:22 PM cells removed from incubator

4:42 pm first PBS spin (including sc's)

4:00 @ 4:56 pm

Hot sc's @ 5:06 pm \rightarrow 5:36 pm Rbc lysis @ 5:39 pm

Cold sc's @ 5:16 pm \rightarrow 5:46 pm Rbc

Hot 37 $^{\circ}$ @ 5:24 \rightarrow 5:54 pm 65 μ l - into same spin

Hot sc's chilling bridge @ 5:57 pm

Take out CD161-Tet sc's and place w/ samples next to

6:11 pm \rightarrow 10 min tetramer head starts

43 $^{\circ}$ @ 6:24 pm \rightarrow 6:54 pm [RBC lysis]

Sc's Fix Perm @ 6:28 pm \rightarrow 38 pm \rightarrow 48 pm

\rightarrow SC Perm Wash (+ tetramer sc wash) @ 6:53 pm

Samples @ 7:14 pm \rightarrow 24 \rightarrow 34

Sc's Intact @ 19:35 \rightarrow 20:15 \rightarrow Done @ 8:09

Samples 7:54 pm Intact @

Done 8:45 pm

3ml + 100 μ l
95 μ l
vial each
excess each
 \rightarrow CD25
CD8 α 1.5
2000 1.5
old PMA
TNTA
5 PM
CD107a stained
near PMA

Unstained cold
 \rightarrow generic
unstained

Unstained hot
 \rightarrow Monocyte
unstained

#	Filter	Single color (ul)	Re ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim III	3	U/D 15 min (RT)	Tetramer 40 min @ RT	HostStain 30min @37C	3	ColdStain 30min @4C	3	RBC Lys, then FixPerm	Spiked 30 min @RT	3
1	UV2				BUV395	CD62L	(DREG-56)								1.2	3.6			
2	UV7				AF	AT-LUV6													
3	UV9				BUV496	CD8	(BPA-18)								0.7	2.1			
4	UV10				BUV653	CD69	(HNS0)								0.5	1.5		1	3.0
5	UV11				BUV615	CCR4	(1G1)												
6	UV4				BUV661	Vβ2	(86)								0.7	2.1		0.5	1.5
7	UV16				BUV737	CKCR3	(CCKR3)												
8	UV1				BUV805	GD4	(SK3)												
9	UV3				BUV421	CD127	(A019D5)											0.5	1.5
10	UV3				Pacific Blue	CD14	(M512)								2.0	6.0			
11	V7				BUV480	CD19	(H1819)								2.0	6.0			
12	V10				BUV510	CD161	(H100)								0.7	2.1			
13	V11				BUV605	CD56													
14	V13				BUV650	CCR7													
15	V14				BUV711	CD7												1	3.0
16	V15				BUV750	IFNγ	(922)												
17	B2				BUV786	CCR6	(11A9)											1.5	4.5
18	B4				AlexaFluor 488	hCD1d													
19	B6				Spark blue 550	CD3	(SK7)												
20	B8				PE	NKG2D													
21	B10				PE-CF594	CD26	(M-A261)											0.5	1.5
22	B13				PE-CF594	CD25	(M-A251)											0.5	1.5
23	B13				PE-CF594	TNFR	(MAB11)											0.5	1.5
24	R1				APC	PD1												2.5	7.5
25	R2				AlexaFluor647	bMFI												0.5	1.5
26	R4				APC-R700	CD107a	(H4A3)												
27	R6				Zombie NIR	U/D													
28	R7				APC/Fire 750	CD27	(O323)												
29	R8				APC/Fire 810	CD38	(H17)												
Antibody Total										6.0	18	Antibody Total	19.0	57	13.7	41.1		9	27
R10 Media										14.5	43.5	Brilliant Stain	50	150	50.0	150		50	150
Pipette draw volume /sample										19.5		Pipette draw volume /sample	66		60.7			56	

Notes:

Simplified Protocol

Thaw cells, DNase, count.
Collect, count, aliquot cells 3.0E6 Cells RT
Bring volume up to 1 ml R10, add 2 ul PMA/C
Cap and incubate at 37 C for 6 hours

Wash with 2 ml PBS, spin down 1300rpm 8m
800 ul of Live/Dead mix (1:2500) @RT for 15m
Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8m
Add HostStain mix, incubate @37C for 30 min
Wash 2 ml 5% PBS-FBS 1300 rpm, 8 min

Add Tetramers, incubate @RT for 10 min
Add ColdStain mix (1:2500) @RT for 15m
Add 300-500 ul 1x RBC Lysis for 3 minutes
Wash 2 ml 5% PBS-FBS 1300 rpm, 8min

300 ul BD FixPerm, incubate @ 4C for 20min
(vortex every

First PermWash: 1 ml PermW/
Second Perm Wash: 1 ml PermW/

Add Intracellular Stain, incubate @ RT for 4C
First PermWash: 2 ml PermW/

Resuspend in 100 ul 0.4% PFA-PBS
Cap tubes, wrap rack in foil, store at 4°C

New	Al CO ₃	TNF	IPN ₅
		15	8.5
	Vd ₂	17.5	23.1

old	CO ₃	15.3	7.3
	Vd ₂	11.6	15.7

Current	CO ₃	14.4	7.8
	Vd ₂	11.3	15.5

Perm		16.2	24.8
	New	23.4	42.2
		22.6	20.7
		20.8	23.0

1/11/2023

[illegible]

Highly purified Stydybeased
Enchotrans

SEB BT202 1mg

11117492aa311