

Sept 23rd
August 17th, 2022

$\gamma\delta$ SFC Panels #2

1:100

380 μ l
153 μ l

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	1.5E+6	0.5 E+6
NDO 27	Adult		10E6	4/4/07		3 ml	1E6	1.73 1.63E6	3		
NDO 27	Adult		10E6	4/4/17		3 ml	.9E6	1.41E6	2.7		

10:47 cont stain starts
10:54 cont stain ends

1) CD16AF350 (4 μ l)
CD27 BVU (1.5 μ l)
+ backbone

4:17 pm \rightarrow 1:32 pm

- 2) SC CD16 (4 μ l)
- 3) SC CD27 (1.5 μ l)
- 4) Unstained SC x2 low signal
- 5) CD45RA SC \rightarrow high signal \downarrow

20 \times
5 μ l media
500

5 vials

Tetramer

1:44 : 2:25

6:40 pm new wellsheet

6:53 pm sample running

4 $^{\circ}$ C Sels @ 2:32 pm \rightarrow 3:02 pm

37 $^{\circ}$ C added @ 2:37 pm \rightarrow 3:07 pm

3:03 \rightarrow 3:06 RBC lyse other controls

3:20 pm \rightarrow 3:50 pm 4 $^{\circ}$ C stain \rightarrow 3:51 pm RBC lyse \rightarrow 3:54

3:21 pm Fix Perm Sels \rightarrow 3:31 \rightarrow 3:41 pm
Sels first perm wash

4:05 Fix Perm \rightarrow 4:15 \rightarrow 4:25

4:48 pm \rightarrow 5:28 pm Intracellular

5:35 pm stains done

#	Filter	Single color (u)	Ref ctrl	Unlinking ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim III	4	L/D 15 min (RT)	Tetramer (RT) for 40 min	37°C (RT) for 30 min	4	Surface staining 40°C for 30 min	4	Using 40 min Cytocor/pe	Intra mix for 40 min	4
1	UV2				BUV995	CD92L	DRF5-56								1.2	48			
2	UV7				Allophycorin 550	CD16									0.7	28		0.25	100
3	UV9				BUV496	CD8	8P4.18								1.0	40		0.25	100
4	UV11				BUV563	CD69	FN50								0.7	28			
5	UV16				BUV615	VE2	(B6)								1.5	80		0.25	100
6	UV3				BUV737	CD27									1.5	80			
7	UV5				BUV805	CD4	SK3								1.5	80			
8	UV7				Pacific Blue	CD14	(M5E2)								1.0	40			
9	UV10				Pacific Blue	CD19	(H1B19)								2.0	80			
10	UV11				BUV480	CD161	(REA631)								2.0	80			
11	UV13				BUV510	CD45RA	(H1100)								1.0	40			
12	UV15				BUV605	CD56									0.7	28			
13	B2				BUV711	CD127									0.7	28			
14	B3				BUV786	CCR6	(11A9)								0.0	00			
15	B4				Allophycorin 488	hCD1d									1.2	48			
16	B6				Spark blue 550	CD3	(SK7)								1.2	48		0.25	100
17	B8				PE	NG2D									0.7	28			
18	B10				PE-CF594	CD26	(M-A261)								1.2	48		0.25	100
19	B13				PE-CF594	CD25	(M-A251)								1.2	48		0.25	100
20	R1				PerCP-eFluor7	Perforin	(d49)								1.5	60		2	800
21	R2				PE-eFluor70	PD1									1.5	60			
22	R4				APC	NG2A									1.0	40			
23	R6				Allophycorin 647	hMIR1									1.5	60			
24	R7				APC-R700	CD107a	(H4A3)								1.0	40			
25	R8				Zombie NIR	L/D									1.5	60			
26	R9				APC-Fire250	CD28									1.5	60			
27	R10				APC-Fire810	CD38	(HIT2)								1.5	60			
And UNSTAINED CONTROLS III																			
Antibody Total									6.8	27.2			5.6	22.4	19.6	78.4		3.25	13
R10 Media									13.7	54.8			50	200	50.0	200		50	200
Pipette draw volume /sample									19.5				55		68.6			52.25	

Simplified Protocol

Aliquot cells 2.0E+6 Cells/tube
 Raise R10 + cell volume to 500 ul
 Add 500 ul of PMA-concomit/Cyt
 Incubate 6 hours @ 37C
 Wash with 2 ml PBS, 1400 rpm, 6min

800 ul Live/Dead @ RT for 15min
 Wash 2 ml 5% PBS-FBS 1400 rpm, 6min
 Add tetramers, incubate @ RT for 40min
 Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

Add hot Surface mix, incubate @ 37C for 30 min
 Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

Add cold Surface mix, incubate @ 4C for 30min
 Add 300-500ul 1x RBC lysis for 3 minutes
 Wash 2 ml 5% PBS-FBS 1500 rpm, 6min

300 ul Cytocor/perm, incubate @ 4C for 20min
 Wash once w/ 2ml Perm Wash
 Resuspend 100 ul 0.4% PFA-PBS, store 4C

Add Intra Staining, incubate @ RT for 40min
 Wash once w/ 2ml Perm Wash
 Resuspend 100 ul 0.4% PFA-PBS, store 4C

SC5 16-74
 27-1.5?
 RA-70.7

011

011

9/23/2022

[illegible]

DATE	DESCRIPTION	AMOUNT	CHECK NO.	BANK	INITIALS
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3-31-78

August 17th, 2022

$\gamma\delta$ SFC Panels #1

Unmixing WIS/16 bottoming out

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$\gamma\delta$ SFC Panels #1

