

- DAVASET  
 360 = 111  
 242

February 01, 2023

450 cpl  
L403

280 cpl  
L403

260 cpl  
L403



2

Ver7.2 ctrl

NJ006  
NJ062

Ver7.2 PMA

2 pl PMA 1000 pl

1 pl PMA 500 pl

1 pl PMA 1000 pl

2 pl old PMA 1000 pl

Zambia CD89 IFN $\gamma$  TNF $\alpha$

NJ062 as sc's

↓

"not great gels"

2 ml

1.83 = 7.96

1:10 5 3 1

○ ○ ○ ○

○ ○ ○ ○

$\times 16 \text{ ctrl tubes} \times 2 = 32$   
 $\frac{200}{32.00}$

$$\begin{array}{r} 5 \times 2 = 10 \text{ pl} \\ \times 200 \\ \hline 1000 \end{array}$$

titration  
1:10 (1) had  $\frac{1}{10}$ th volume removed  $2 \times 200$   
to make standard/sols for NDOCE Vol 2

5-OPRU	1:10	1/1	10 → 5, 3, 1
6-FP	1:10	1/1	10 → 5, 3, 1
hcdh	1:10	1/1	10 → 5, 3, 1
sob	1:10	1/1	10 → 5, 3, 1

8 tubes \* 0.3  $\mu$ L of acid  
3  $\mu$ L in 27  $\mu$ L

RBC lysis @ 20:40pm →

200  $\mu$ L RBC lysis (1 mL FB / PBS)

1000

1000

1000

1000

1000

1000

1000

February 8th, 2023

PMA: Kitchen Sink

Hot scs & Hot Samples spin @ 1400 6min  
(RBCs) 2ml @ 8:58pm →

Cold scs out @ 9:08 pm / RBC lysis @ 21:10 pm

Scs in fridge @ 9:25 pm spin @ 21:14

Tetramers @ 9:42 pm → 10:22 pm

Sc tets @ 21:48 pm → 22:03 pm (spin @ 10:06 pm)

Sc's FixPerm @ 21:53 → 03 → 13 pm 1st wash @ 10:18 pm

Sc tets FixPerm @ 10:18 → 28 → 38 pm

Samples + scs  
(1st wash) (2nd Perm wash) @ 10:32 pm // 10 min wait //

59 / 53.5

4°C @ 10:50 pm → 11:20 pm / RBC lysis @ 11:21 pm

Bulk scs @ 11:00 w/ 40 µl 0.4% AF8-B5

Sc's intra @ 11:07 → 11:47 pm / spin w/ 2nd Perm wash

Samples RBC/4°C spin @ 11:47 pm ✓

11:38 pm → 48 → 58 pm

Sc's done @ 12:07 am //

Samples 2nd Perm wash @ 12:14 am

54.5 / 48 Intracellular @ 12:27 → 1:07 pm

Final spin @ 1:09 am

Done @ 1:21 pm

Acquisition 8:23pm on 02/09/2023 (Aurora 5L install hiccups  
disc/conn repeatedly)  
event rate 1000 cells for chds. (12000 events/50  $\mu$ L)  
CD69 1500 events

SSC-A glitch is back  $\wedge$  CD14

TEMRA (CD45RA + CCR7  $\bar$  CD27-) CD8+ T cells

Set up PeacoQC script for sc evaluation?

~~★~~ NY62D

Cord Zombic actually ~~★~~ Adult Zombic

~~★~~ Unstained scs Hot NY62 & monocytes

// Scs done @ 10:13pm (unacceptable)

Samples @ 10:21 pm ~~★~~

~~★~~ ND006 appears fragile  
(monocyte cloud vanished) ~~★~~

Graph not concentrated,  
Useful analysis talk  
about  $\bar$  expen.

Coefficients as comparisons



#	Filter	Single color (u)	Ref ctrl	Unmixing dt	Fluorescence	Marker	Clone	Vial Lot #	During stim	8	L/D 15 min (RT)	Tetramer 40 min @ RT	HostStain 30min @37C	8	ColdStain 30min @4C	8	RBC Lysate then Fcy/Perm	Spiked 30 min @RT	8
1	UV2				BUV995	CD62L	(DMEG-56)								1.2	9.6			
2	UV7				AF	AP-UV6													
3	UV9				BUV995	CD8	(RPA-18)								0.7	5.6			
4	UV10				BUV661	CD69	(EJ50)								0.5	4.0		1	8.0
5	UV11				BUV661	CCR4	(1G1)						2.0						
6	UV4				BUV737	Vβ2	(B6)							16	0.7	5.6		0.5	4.0
7	UV16				BUV995	CCR3	(SK3)								1.3	10.4			
8	UV1				BUV995	CD4	(A03905)												
9	UV3				Pacific Blue	CD127	(MSE2)						1.5	12				0.5	4.0
10	UV5				Pacific Blue	CD14	(H1B19)						1.5	12					
11	V7				BUV480	CD19	(BEA631)						2	16	2.0	16.0			
12	V10				BUV510	CD45RA	(H1100)								0.7	5.6			
13	V11				BUV405	CD56							1.0	8					
14	V13				BUV510	CCR7							1	8				1	8.0
15	V14				BUV750	IFNγ	(B27)						1	8					
16	V15				BUV786	CD68	(11A9)						1.5	12				1.5	12.0
17	B3				AlexaFluor 488	hCD1d													
18	B4				Spart blue 550	CD3	(SK7)												
19	B6				PE	NKG2D									1.2	9.6		0.5	4.0
20	B8				PE-Cy594	CD26	(M-A261)						1.5	12				0.5	4.0
21	B10				PE-Cy5	CD25	(M-A261)						1.2	9.6					
22	B13				PerCP-Cy5.5	TNfr	(MAB11)											0.5	4.0
23	R1				PE-wo770	PD1												2.5	20.0
24	R2				AlexaFluor 488	CD16									1.5	12.0		0.5	4.0
25	R4				APC-FITC	Vβ2									0.7	5.6			
26	R6				Zombie NIR	I/D	(H4A3)								1.2	9.6		0.5	4.0
27	R7				APC/Fire 750	CD27	(O323)												
28	R8				APC/Fire 810	CD38	(H172)												
And UNSTAINED CONTROLS !!!																			
Antibody Total									6.0	48	Antibody Total	19.0	152	13.7	109.6	9.5	76		
R10 Media									14.5	116	Brilliant Stain	50	400	50.0	400	50	400		
Pipette draw volume /sample									19.5		Pipette draw volume /sample	66		60.7		56.5			

Notes:

## Simplified Protocol

Thaw cells, DNase, count.  
Collect, count, aliquot cells 3.0E+6 Cells R10 / 5ml polystyrene tube  
Bring volume upto 1 ml R10, add 2 ul PMACr1 and CD107 a  
Cap and incubate at 37°C for 6 hours

Wash with 2 ml PBS, spin down 1300rpm 8min  
800 ul of Livebead mix (1:2500) @RT for 15min  
Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min

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

Add Tetramers, incubate @RT for 10 min  
Add ColdStain mix, incubate @4°C for 30min  
Add 300-500 ul 1% RBC Lysis for 3 minutes  
Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

300 ul BD FACSPerm, incubate @4°C for 20min  
(vortex every 10 minutes)

First PermWash: 1 ml PermWash 1500 rpm 6 min  
Second Perm Wash: 1 ml PermWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min  
First PermWash: 2 ml PermWash 1500 rpm 6 min

Resuspend in 70 ul 0.4% PFA-PBS  
Cap tubes, wrap rack in foil, store at 4°C

CD35 → 1.2  
Biotin → 2.0 PL



February 8th, 2023

1:100 Titrations

#	Filter	Single color (u)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	8	L/D 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @37C	8	ColdStain 30min @4C	8	RBC Lyse, then Fix/Perm	Spiked 30 min @RT	8
1	UV7				BUV496	CD8	(RPA-T8)												
2	UV11				BUV661	V62	(B6)								0.7	5.6			
3	UV16				BUV805	CD4	(SK3)								0.7	5.6		0.5	4.0
4	V3				Pacific Blue	CD14	(M5E2)						1.5	12				0.5	4.0
5	V3				Pacific Blue	CD19	(H1B19)								2.0	16.0			
6	V5				BUV480	CD161	(REA531)						2	16	2.0	16.0			
7	V10				BUV510	CD45RA	(H1100)								0.7	5.6			
8	V11				BUV605	CD56							1.0	8					
9	V13				BUV711	CCR7							1	8				1	8.0
10	B2				AlexaFluor 488	hCD1d							1	8					
11	B3				Spark blue 550	CD3	(SK2)					< : > ( )			< : > (1.2)				
12	B4				PE	NKG2D									1.2	9.6		0.5	4.0
13	R1				APC	CD16							1.5	12				0.5	4.0
14	R2				AlexaFluor647	Va7.2									0.7	5.6			
15	R6				Zombie NIR	L/D						< : > ( )			1.2	9.6		0.5	4.0
Antibody Total										0.0	0	Antibody Total	8.0	64	9.2	73.6		3.5	28
R10 Media										20.5	164	Brilliant Stain	50	400	50.0	400		50	400
Pipette draw volume /sample										19.5		Pipette draw volume /sample	55		56.2			50.5	
And UNSTAINED CONTROLS !!!																			

Notes:

- 1.2

- 0.5





## 2/1/2023

[illegible]



February 8th, 2023

PMA: Kitchen Sink



February 8th, 2023

PMA: Kitchen Sink



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१५ : ०५ : १५