January 4th, 2023

Tetramer Staining Order 1.5

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
NY062 (1)	Adult		20E6	08/12/21		2	4.49	6.15	8.89		
NY062 (2)	Adult		20E6	08/12/21		2	6.19	8.32	12.38		
							5.34		21.36	.280µl	
										+220µl	

300µl/40µl

350µl/40µl

Thaw <9:10 – 9:50 am (after PPD cells)>
10:08 am Stain count

Incubation @ 11:36 am → 5:36 pm

PMA, Ctrl Blood, + CD107a respectively

T+H+C | H+T+C | H+C+T | HT+C ✓ | 14+CT | Va7.2 | Va24Ja18 1.2µl

PMA ○ ○ ○ ○ ○ ○ ○

ctrl ○ ○ ○ ○ ○ ○ ○

130µl / ? = 280µl / 500µl → only 130µl unstained tube

232 = 500 / 1µl → 0.466µl PMA

Add individually

Not following tetramer controls today just staining order for fetl

do a barebone mix

15:35 pm <Abs prepped>

All mixes done @ 5:25 pm

Spin down @ 6:57 pm

- CD107a sc
  - CD8, CD45RA, IFNγ, TNFα, CD69 sc PMA
  - CX3CR3, NKG2D, Filter issue ctrl
    ?not enough for a signal ... beads?

6:11 Live Dead → 6:24 pm ✓

scs @ 6:18 pm → 48 pm

Spin @ 6:30 pm

10 tests of hMR1 (new)
<0.5>
6µl in 95µl ~ 5µl
<11µl>

10 test of hCD1d

10 test of hCD1d (new)
<0.3>
<4µl in 36µl> ~ 3µl

both in @ respective tetramer timepoints

22 × .8 = 19.6

2 19.1 × 4 = 78.3

Tets @ 6:48 pm

1st Hot Wave in @ 6:59 → 7:30 pm

Sc's RBC lysed ✓

2/5 8pt-20 9pt-22.5mls 4D

7:30 grab hots & RT tets...

SC's @ 7:08pm → 18 → 28
FixPerm

7:39pm Samples FBS spin 1300 rpm 8 min (no speed!!)
+ SC's 2nd Perm Wash.

2nd tetramer wave @ 7:56pm H-T-c & H-Tc

Intrac SC's @ 20:04pm → 8:44pm

→ 20:11 → 8:41 Hot/cdd/RT 30 min

2nd wave spin @ 20:48 pm

- iNKT & Va7 didn't get tetramers @ end cold... ☆ so just SC controls equivalents ☆
  + HTc & HTc Fix Perm @ 21:04 → 19 → 24pm ✓
  Into spin into 2nd spin @ 9:38
- HcT into tet @ 21:06 → 21:46 pm
  final Cold Tets @ 21:08 → :38 pm

First sets Intrac @ 21:55 pm → 10:35pm

2nd batch FixPerm @ 10:03 pm → ~~:13~~ :23

→ 1st perm wash @ :13 2nd @ 22:38 3rd wash @ 22:38

Intrac 54.5µl (reg order + restained) → @ 22:57 → 11:37pm

4th wash
HTc & HTc
Spin @ 10:55pm

Final wash @ 11:51pm → Done @ 00:01

into 3rd perm Wash @ 11:42pm

Fixed w/ 50µl 0.4% PFA-PBS ~23:10pm

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H-Tc PMA
Va7.2 PMA

Tet-c ctrl
H-Tc ctrl

↑ 2µl CD107a in Jan5th - PMA looks fairly weak ...

Monocytes picked up the ab

——— cells
——— Beads
——— (new)

Template (duplicate) w or w/o data

January 4th, 2023 Tetramer Staining Order

#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	11	L/D 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @37C	11	ColdStain 30min @4C	11	RBC Lyse, then Fix/Perm	Intracellular Stain 40 min @RT	11
1	UV2				BUV395	CD62L	(DREG-56)								1.2	13.2			
2	UV7				AF	AF-UV6													
3	UV9				BUV496	CD8	(RPA-T8)								0.7	7.7			
4	UV10				BUV563	CD69	(FN50)								0.5	5.5		1	11.0
5	UV11				BUV615	CCR4	(1G1)						2.0	22					
6	U14				BUV661	Vδ2	(B6)								0.7	7.7			
7	UV16				BUV737	CXCR3	(1C6/ CXCR3)								2.5	27.5			
8	V1				BUV805	CD4	(SK3)						1.5	16.5				0.5	5.5
9	V3				BV421	CD127	(A019D5)						1.5	16.5					
	V3				Pacific Blue	CD14	(M5E2)								2.0	22.0			
10	V5				Pacific Blue	CD19	(HIB19)								2.0	22.0			
11	V7				BV480	CD161	(REA631)						2	22					
					BV510	CD45RA	(Hi100)								0.7	7.7			
12	V10				BV605	CD56							1.0	11					
13	V11				BV650	CCR7							1.5	16.5				0.5	5.5
28	V13				BV711	CD7							1	11					
14	V14				BV750	IFNγ	(B27)											1.5	16.5
15	V15				BV786	CCR6	(11A9)						1.5	16.5					
16	B2				AlexaFluor 488	hCD1d						< : > ( )			1.2	13.2			
17	B3				Spark blue 550	CD3	(SK7)						1.5	16.5				0.5	5.5
18	B4				PE	NKG2D							1.2	13.2				0.5	5.5
19	B6				PE-CF594	CD26	(M-A261)						1.2	13.2				0.5	5.5
20	B8				PE-Cy5	CD25	(M-A251)						1.2	13.2				2.5	27.5
21	B10				PerCP-Cy5.5	TNFα	(MAB11)								1.5	16.5		0.5	5.5
22	B13				PE-vio770	PD1									0.7	7.7			
	R1				APC	CD16						< : > ( )							
23	R2				AlexaFluor647	hMR1													
24	R4				APC-R700	CD107a	(H4A3)		2.0	22									
25	R6				Zombie NIR	L/D					<1:2500>								
26	R7				APC/Fire 750	CD27	(O323)						2	22					
27	R8				APC/Fire 810	CD38	(HIT2)						1.6	17.6					
And UNSTAINED CONTROLS !!!						Antibody Total			2.0	22	Antibody Total		19.5	215	13.7	150.7		7.5	82.5
						R10 Media			18.5	204	Brilliant Stain		50	550	50.0	550		50	550
						Pippette draw volume /sample			19.5		Pippette draw volume /sample		67		60.7			54.5	

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 PMA/Ctrl and DuringStim antibody
Cap and incubate at 37°C for 6 hours

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

Add tetramers for 40 minutes at RT
Wash with 2 ml PBS, spin down 1300rpm 8min

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

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

300 ul BD FixPerm, incubate @ 4C 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: 1 ml PermWash 1500 rpm 6 min
Second Perm Wash: 1 ml PermWash 1500 rpm 6 min

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

ILT & NK SFC Panel

1/4/2023

Spectrum	UV			Violet			Blue			Red			
373	UV1												
388	UV2	BUV395	[2] CD62L										
428	UV3			V1	BV421	[4] CD127							
443	UV4			V2									
458	UV5			V3	PacBlue	[1] CD14/19			Vd24				
473	UV6	AF-UV6	AF	V4					~~Vd25~~				
508				V5	BV480	[3] CD161	B1		~~NKT~~				
514	UV7	BUV496	[2] CD8					Fitc					
525				V6			B2	~~AF488~~	[1.5] ~~hCD1d~~				
542	UV8			V7	BV510	[1.5] CD45RA	B3	SparkBlue 550	[1] CD3				
582	UV9	BUV563	[3] CD69	V8	BV570		B4	PE	[4] NKG2D				
598				V9			B5						
613	UV10	~~BUV615~~	[3] ~~CCR4~~	V10	BV605	[3] CD56	B6	PE-CF594	[4] CD26				
664	UV11	BUV661	[3] Vd2	V11	BV650	[3.5] CCR7	B7			R1	APC	[3.5]	CD16
679							B8	~~PE-Cy5~~	[4.5] ~~CD25~~	R2	AF647	[3.5]	hMR1
697	UV12			V12			B9	PerCP-Cy5.5	[2] TNFa	R3			
717	UV13			V13	BV711	[4] CD7	B10			R4	APC-R700	[3]	CD107a
738							B11			R5			
750	UV14	~~BUV737~~	[3] ~~CXCR3~~	V14	BV750	[2.5] IFNg							
760							B12			R6	Zombie NIR	[ ]	L/D
783	UV15			V15	BV786	[3] CCR6	B13	Pe-Vio770	[3] PD1	R7	APC-Fire 750	[2]	CD27
812	UV16	BUV805	[1] CD4	V16			B14			R8	APC-Fire 810	[3]	CD38

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Unmixing: 0.98 similarity ctrl vs PMA lymphocytes
.99 ctrl vs PMA monocytes.

hMR1/Va7.2+ cluster
___ % ___

0.88 H-C-T
0.82 H-T-C
0.098 H-TC
0.044 T-H-C
0.052 TH-C
0.21 Va7.2

CD4? DN?

included Va7.2

CD3+ CD161+
PacMAP of 182,228 cells

Va7.2+ ~~703~~ 550/703 ≈ .782

1 THC : 170/751 ≈ .226
2 H-T-C : 334/~~839~~ ≈ .398
3 H-C-T : 194/726 ≈ .250
4 HT-C : 196/726 ≈ .269
5 H-TC : 446/1088 ≈ .409

15

No hMR1 dump
viability or AF

Va7.2 15,6950 cells
CD4- PacMAP → 3900
concat → 30 TPZ

Va7.2 ~ 1.000
THC ~ 0.289
HTC ~ 0.508
HCT ~ 0.319
HT-C ~ 0.343
H-TC ~ 0.523

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