April 15th, 2023

TNFa Location

12904μl

250μl → TSK

Specimen	Status	Location	Conc	Date	Tasks	Volume	(1:100) Ly EC	Ly+Mon	Total	2E+6	1.5 3E+6
Inf142 a-7	Cord S76L		<7.4μl>			2	7.74	10.9	15.48	260μl	190μl

2:05 pm Thaw
2:14 pm Spin
2:34 stain
2:47 count start
Incubation @ 3:13pm → 9:13pm
Ab prep @ 8:30pm
Done w/ prep @ 9:10pm
Spin @ 9:21pm
L/D @ 9:35 pm
Spin @ 9:54pm 63μl
22:07pm Hot Samples @ → 22:37 pm
Vial's @ 22:50 → 11:20

7 ×800 / 560μl

Cold stain @ 23:33 → 00:03am (57.5)
Lyse @ Spin
Fix Perm @ 00:19 am → 29 am → 39 am
1st wash @ 12:43pm
2nd @ 12:53 am
Headstart TNFa @ 1:06 am (15 for Dazzle, CF594; 30 for PerCP-Cy55)
1:19 am Intracellular → 2:00 Am
(ctrl Per-CP-Cy5.5 didn't get, messed up calcs?)
Spin @ 2:00 am (30/45μl 0.4)
← angry grumbles
Done 2:10am

	unst	CF594	Dazzle	PerCP-Cy55
PMA	O	O	O	O
ctrl	O	O	O	O

2M cells each

IF594 Dazzle PerCP-Cy5.5
O O O

O CD107a

~~CF594~~ O Zombie   O new CCR4

Cells 260μl
R10 358μl

50μl * 1.32 × 4 → 205μl R10

5.28μl of stim

4μl of CD107a

+ 0.1 CD56   intraspike +0.1 CD3

20.5 − 2.7 = 17.8 × 6 = 106.8
1.5 × 6 = 9
1.2 × 6 = 7.2

71.4μl
amm

April 15th, 2023

TNFa Location

Sample	Total	Volume	Concentration	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF142	15.48	2	7.74	0.39	0.36	0.32	0.29	0.26	0.23	0.19	0.16	0.13	0.10	0.06
NA	5	1	5.00	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10
NA	5	1	5.00	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10
NA	5	1	5.00	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10
NA	5	1	5.00	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10

	Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
R10	INF142	0.61	0.561	0.510	0.46	0.408	0.357	0.31	0.255	0.204	0.15	0.102
	NA	0.40	0.366	0.333	0.30	0.266	0.233	0.20	0.166	0.133	0.10	0.067
	NA	0.40	0.366	0.333	0.30	0.266	0.233	0.20	0.166	0.133	0.10	0.067
	NA	0.40	0.366	0.333	0.30	0.266	0.233	0.20	0.166	0.133	0.10	0.067
	NA	0.40	0.366	0.333	0.30	0.266	0.233	0.20	0.166	0.133	0.10	0.067

	Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
PMA	INF142	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	NA	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	NA	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	NA	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	NA	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33

	Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M
CD107a	INF142	6	5.5	5	4.5	4	3.5	3
	NA	6	5.5	5	4.5	4	3.5	3
	NA	6	5.5	5	4.5	4	3.5	3
	NA	6	5.5	5	4.5	4	3.5	3
	NA	6	5.5	5	4.5	4	3.5	3

April 15th, 2023 TNFa PE-CF594 PE-Dazzle PerCP-Cy5.5

#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	6	L/D 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @37C	6	ColdStain 30min @4C	6	RBC Lyse, then Fix/Perm	Spiked 30 min @RT	6
1	UV2				BUV395	CD62L	SK11								1.2	7.2			
					AF	AF-UV6													
2	UV7				BUV496	CD8	RPA-T8								0.7	4.2			
3	UV9				BUV563	CD69	FN50								0.5	3.0		1	6.0
4	UV10				BUV615	CCR4	1G1						2.0	12					
5	UV11				BUV661	Vδ2	B6								0.7	4.2		<0.25>	######
6	U14				BUV737	CXCR3	1C6/CXCR3								1.3	7.8			
7	UV16				BUV805	CD4	SK3						1.5	9				0.5	3.0
8	V1				BV421	CD127	A019D5						1.5	9					
9	V3				Pacific Blue	CD14	M5E2								2.0	12.0			
	V3				Pacific Blue	CD19	SJ25C1								2.0	12.0			
10	V5				BV480	CD161	HP-3G10						2	12					
11	V7				BV510	CD45RA	HI100								0.7	4.2			
12	V10				BV605	CD56	5.1H11						1.0	6					
13	V11				BV650	CCR7	G043H7						1	6				1	6.0
14	V13				BV711	CD7	M-T701						1	6					
15	V14				BV750	IFNγ	B27											1.5	9.0
16	V15				BV786	CCR6	11A9						1.5	9					
17	B2				FITC/AF488	Va24/hCD1d	6B11					<5/100>			<1.5>			<0.25>	
18	B3				Spark blue 550	CD3	SK7								1.2	7.2		0.1	0.6
19	B4				PE	NKG2D	1D11						1.5	9				0.5	3.0
20	B6				PE-CF594	CD26	M-A261						<1.2>	####					
21	B8				PE-Cy5	CD25	M-A251						1.2	7.2				0.5	3.0
22	B10				PerCP-Cy5.5	TNFα	MAb11											<3.0>	######
23	B13				PE-vio770	PD1	PD1.3.1.3								1.5	9.0		0.5	3.0
24	R1				APC	CD16	3G8								0.7	4.2			
25	R2				AlexaFluor647	Va7.2/hMR1	3C10					<5/100>			<1.2>			<0.25>	
26	R4				APC-R700	CD107a	H4A3		6.0	36									
27	R6				Zombie NIR	L/D	N/A				<1:2500>								
28	R7				APC/Fire 750	CD27	O323						2	12					
29	R8				APC/Fire 810	CD38	HIT2						1.6	9.6					
							Antibody Total		6.0	36	Antibody Total		17.8	107	12.5	75.0		5.6	33.6
And UNSTAINED CONTROLS !!!							R10 Media		14.5	87	Brilliant Stain		50	300	50.0	300		50	300
							Pippette draw volume /sample		19.5		Pippette draw volume /sample		65		59.5			52.6	

Simplified Protocol

Thaw cells, DNAse, count.
Collect, count, aliquot cells 2-3.0E+6 Cells F
Bring volume upto 1 ml R10, add 2 ul PMA/I
Cap and incubate at 37°C for 6 hours

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

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

Add Tetramers, incubate @RT for 10 min
Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min

Add ColdStain mix, incubate @ 4C for 30mi
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 20m
(vortex ever

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

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

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

56 +37

20.5
- 8.6
11.9 µl × 6 = 71

ILT & NK SFC Panel

4/15/2023

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							
473	UV6	AF		V4									
508				V5	BV480	[3] CD161	B1						
514	UV7	BUV496	[2] CD8										
525				V6			B2	FITC/AF488	[1.5] Va24/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/Dazzle	[4] TNFa?				
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]	Va7.2/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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