June 29th, 2023

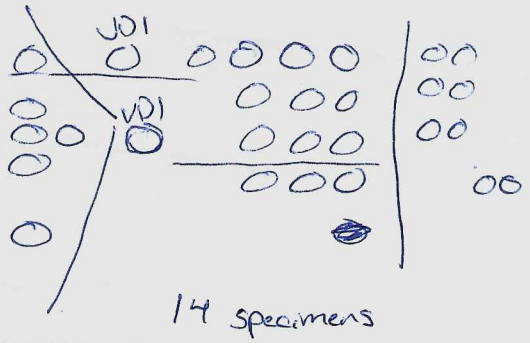
-400 CD107a

2023_ILT_10

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
Inf636-5 a-2	HU ♂ TKQD	18A G6 (2)			lots of cells, all lysed	1	11	11	11
Inf809-1 a-1	U6X6	(10)	<7.53>			1	11.9	14.0	
Inf809-7 a-2	HU ♂ U6X7	22A I1 (1)	<8.17		large clump	1	11.9	15.4	
Inf 665-7 a-3	TLXQ	(9)	<12.5>			0.5	6.96	11.1	
Inf665-7 a-1	HEU-lo ♂ TLXN	19A C4 (4)	<10.97			0.5	7.84	12.2	
Inf665-7 a-2	TLXP	(7)	<11.47			0.5	7.25	11.0	
Inf718-6 a-1	HEU-hi ♀ TVUJ	20A G7 (3)	<12.15>			1	7.25	11.0	
Inf 718-6 a-2	TVUR	(8)	<11.27			1	7.79	11.0	
ND050	Adult ♂	(5)	<11.27	1/13/23		1	9.00	11.8	
ND006	Adult ♀	(6)	<6.47 ISE6	5/12/23		1	15.3	18.3	

UP
1430c. 40 µl
1520c. 40 µl
1300c. 40 µl
1380c. 40 µl
1240c. 40 µl
1200c. 40 µl
1085c. 40 µl
710c. 40 µl
1170c. 40 µl

10:48 am start spin @ 11:05 dnase @ 11:23 resus @ 11:41
start 11:09 spin @ 11:14 dnase @ 11:29 resus @ 11:41
start spin @ 11:36 dnase @ 11:52 resus @
2nd 309 start spin 11:47 dnase resusp



14 specimens

12:17pm stain for count
12:33pm count.
~~1:03~~ aliquot start
1:23
2:00 pma prepped

Incubation Start @ 2:15pm → 8:15pm

Out of PermWash ½ 70mls left, need 140

8:34 pm cells spin
8:51 pm H/D → 9:06pm
~~everything is trying to screw up~~ - centrifuge spinning abs
Spin @ 9:09 pm
Hot samples @ 9:25 → 9:55pm
tets made @ 9:36 (Fresh OP-RU)
Abs & reagents prepped @ 9:54pm
spin @ 9:59 pm

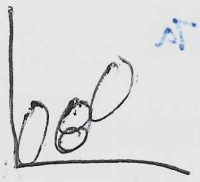
Tets @ 10:16 → 10:56pm
Vd1's & Va's 10:23 → 10:53pm
Sc's aliquot & spin @ 10:45 pm

11:03pm sc 4/0 → 11:18pm
Hots cs @ 11:08pm → 11:36pm (spin @ 41)
Cold samples @ 11:16pm → 46 rm
Cold scs @ 11:27pm → 11:57pm (SA 56)
↳ spin @ 00:01pm
FixPerm (200µl) @ 00:15 → 25 → 35
1st wash (w/ 400µl) @ 00:43
2nd wash @ 1:02am
1:18 am intra / 21 for scs → 1:58 am
1:33 am scs 20µl (deep top)
2:02 am final wash (1.3mls) Done @ 2:18 am

start @ 2:08 pm

Samples @ 3:18 pm

Done by 3:55 pm

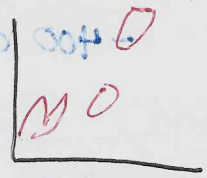


Inf 800

11K/.5K

19K/1.7M

47M



665

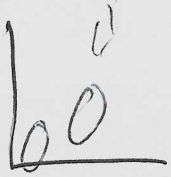
25K/4M

poor vocab.lly?

we had to combine 3 trials after all

30M 17 GK

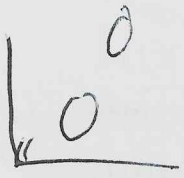
31M 8M



718

71.5/.5il

multiple hits AFs...



ND050

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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
INF636	0	0	#DIV/0!	#####	#DIV/0!	#DIV/0!	#####	#DIV/0!	#DIV/0!	#####	#DIV/0!	#DIV/0!	#####	#DIV/0!
INF809	23.8	2	11.90	0.25	0.23	0.21	0.19	0.17	0.15	0.13	0.11	0.08	0.06	0.04
INF665	11.02	1.5	7.35	0.41	0.37	0.34	0.31	0.27	0.24	0.20	0.17	0.14	0.10	0.07
INF718	15.04	2	7.52	0.40	0.37	0.33	0.30	0.27	0.23	0.20	0.17	0.13	0.10	0.07
ND050	9	1	9.00	0.33	0.31	0.28	0.25	0.22	0.19	0.17	0.14	0.11	0.08	0.06
ND006	15.03	1	15.03	0.20	0.18	0.17	0.15	0.13	0.12	0.10	0.08	0.07	0.05	0.03

R10

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF636	#####	#DIV/0!	#DIV/0!	#####	#DIV/0!	#DIV/0!	#####	#DIV/0!	#DIV/0!	#####	#DIV/0!
INF809	0.75	0.685	0.623	0.56	0.498	0.436	0.37	0.311	0.249	0.19	0.125
INF665	0.69	0.542	0.493	0.44	0.394	0.345	0.30	0.246	0.197	0.15	0.099
INF718	0.60	0.550	0.501	0.45	0.400	0.350	0.30	0.250	0.200	0.15	0.101
ND050	0.67	0.610	0.555	0.50	0.444	0.389	0.33	0.277	0.222	0.17	0.111
ND006	0.80	0.733	0.667	0.60	0.533	0.467	0.40	0.333	0.266	0.20	0.134

PMA

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF636	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF809	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF665	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF718	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
ND050	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
ND006	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33

CD107a

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M
INF636	6	5.5	5	4.5	4	3.5	3
INF809	6	5.5	5	4.5	4	3.5	3
INF665	6	5.5	5	4.5	4	3.5	3
INF718	6	5.5	5	4.5	4	3.5	3
ND050	6	5.5	5	4.5	4	3.5	3
ND006	6	5.5	5	4.5	4	3.5	3

Volume	Lym	Total	Average
1	7.25	7.25	
1	7.79	7.79	**7.52**
		15.04	

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#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	15	L/D 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @37C	15	ColdStain 30min @4C	15	RBC Lyse, then Fix/Perm	Spiked 40 min @RT	15
1	UV2				BUV395	CD62L	SK11								1.2	18.0			
2	UV7				AF	AF-UV6													
3	UV9				BUV496	CD8	RPA-T8								0.7	10.5			
4	UV10				BUV563	CD69	FN50								0.5	7.5		1	15.0
5	UV11				BUV615	CCR4	1G1						2.0	30					
6	U14				BUV661	V82	B6								0.7	10.5		0.1	1.5
7	UV16				BUV737	CXCR3	1C6/CXCR3								1.3	19.5			
8	V1				BUV805	CD4	SK3						1.5	22.5				0.5	7.5
9	V3				BV421	CD127	A019D5						1.5	22.5					
	V3				Pacific Blue	CD14	M5E2								2.0	30.0			
10	V5				Pacific Blue	CD19	SJ25C1								2.0	30.0			
11	V7				BV480	CD161	HP-3G10						2	30					
					BV510	CD45RA	HI100								0.7	10.5			
12	V10																		
13	V11				BV605	CD56	5.1H11						1.0	15				0.1	1.5
14	V13				BV650	CCR7	G043H7						1	15				0.7	10.5
15	V14				BV711	CD7	M-T701						1	15					
16	V15				BV750	IFNγ	B27											1.5	22.5
17	B2				BV786	CCR6	11A9						1.5	22.5					
18	B3				FITC/AF488	Va24/hCD1d	6B11					<:10/1.5>			1.2	18.0		0.1	1.5
19					Spark blue 550	CD3	SK7											2.5	37.5
20	B4				PerCP-Cy5.5	CD26	BA5b						1.5	22.5				0.5	7.5
21	B6				PE	NKG2D	1D11											1.5	22.5
22	B8				PE-Dazzle594	TNFa	MAB11						1.2	18				0.5	7.5
23	B13				PE-Cy5	CD25	M-A251								1.5	22.5		0.5	7.5
24	R1				PE-vio770	PD1	PD1.3.1.3								0.7	10.5			
25	R2				APC	CD16	3G8					<2:10/1.2>							
26	R4				AlexaFluor647	Va7.2/hMR1	3C10												
27	R6				APC-R700	CD107a	H4A3		6.0	90									
28	R7				Zombie NIR	L/D	N/A				<1:2500>								
29	R8				APC/Fire 750	CD27	O323						2	30					
					APC/Fire 810	CD38	HIT2						1.6	24					
							Antibody Total		6.0	90	Antibody Total		17.8	267	12.5	187.5		9.5	142.5
And UNSTAINED CONTROLS !!!							R10 Media		14.5	217.5	Brilliant Stain		50	750	50.0	750		11	165
							Pippette draw volume /sample		19.5		Pippette draw volume /sample		65		59.5			19.5	

Simplified Protocol

Thaw cells, DNAse, count.
Collect, count, aliquot cells 2-3.0E+6 Cells R10 / 5ml polystyrene tube
Bring volume up to "x" mL R10, add "y" µL PMA/Ctrl and "z" µL CD107a
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 HotStain mix, incubate @37C for 30 min
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 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)

2.5 | 40 | 150 | 16

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

Va Mix			8
FITC	Va24Ja18	1.5	12
AF647	Va7	1.2	9.6
	PBS	17.8	142.4
Pippette draw volume/sample:		19.5	

Tetramer Mix			4
AF488	hCD1d PBS-57	0.5	2.4
AF647	hMR1 5-OP-RU	2	9.6

Tetramer Control Mix			1
AF488	hCD1d Unloaded	0.5	0.6
AF647	hMR1 6-FP	2	2.4

Zombie NIR	17		
0.8	13.6	15	PBS
	5.44	6	Zombie

	10	20	30
Tet:PBS	1_9	2_18	3_27
Tet:PBS	1_9	2_18	3_27

	10	20	30
Tet:PBS	1_9	2_18	3_27
Tet:PBS	1_9	2_18	3_27

	Total	FBS	RBC Lyse	Fix	Perm	PFA
Number Samples	15	120	4.5	4.5	60	0.45
Number Unstained	10	40	3	2	20	0.3
Number Surface SCs (29)	29	29	0	5.8	58	0.58
Number Intracellular SCs	6	6	0	1.2	24	0.12
Total		195	7.5	13.5	162	1.45
		175.5			145.8	

FBS	97.5
PBS	97.5

PFA	0.145
PBS	1.305

Perm	16.2
Water	146

RBC	0.75
Water	6.75

ILT & NK SFC Panel

Spectrum	UV			Violet			Blue			Yellow-Green			Red			
373	UV1															
388	UV2	BUV395	[50] CD62L													
428	UV3			V1	BV421	[45] CD127										
443	UV4			V2												
458	UV5			V3	PacBlue	[55] CD14/19										
473	UV6	AF		V4												
508				V5	BV480	[55] CD161	B1									
514	UV7	BUV496	[55] CD8													
525				V6												
542	UV8			V7	BV510	[55] CD45RA	B2	FITC/AF488	[55] Vα24/hCD1d							
582	UV9	BUV563	[55] CD69	V8	BV570		B3	SparkBlue 550	[50] CD3							
598				V9			B4			YG1	PE	[50] NKG2D				
613	UV10	BUV615	[45] CCR4	V10	BV605	[50] CD56	B5									
664	UV11	BUV661	[50] Vδ2	V11	BV650	[50] CCR7	B6			YG3	PE-Dazzle594	[60] TNFα				
679							B7						R1	APC	[55]	CD16
697	UV12			V12			B8			YG5	PE-Cy5	[60] CD25	R2	AF647	[45]	Vα7.2/hMR1
717	UV13			V13	BV711	[55] CD7	B9	PerCP-Cy5.5	[45] CD26				R3			
738							B10						R4	APC-R700	[55]	CD107a
750	UV14	BUV737	[40] CXCR3	V14	BV750	[50] IFNγ	B11						R5			
760																
783	UV15			V15	BV786	[50] CCR6	B12						R6	Zombie NIR	[55]	Viability
812	UV16	BUV805	[50] CD4	V16			B13			YG9	Pe-Vio770	[50] PD1	R7	APC-Fire 750	[45]	CD27
							B14						R8	APC-Fire 810	[50]	CD38

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