July 3rd, 2023

2023_ILT_11

(margin)	Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
2030 c. 60µl	Inf0280 a-2	HU ♂ RR7R	(5)	<10.28>		LN2 inside	1	5.81	10.4	
2050 c. 60µl	a-1	RR7Q	(11)	<9.84>			1	6.15	11.1	
40µl	Inf939-1 bx-3?	HU ♂ V8LC	(1)			lysed? ~~res final~~ Lysed :(	1	~~X~~	~~X~~	
40µl	bx-2	V8LB	(9)			Lysed :(	1	~~X~~	~~X~~	
40µl	Inf636-5 a-1	HU ♂? TK9C	(2)			lysed? res final Lysed :(	1	~~X~~	~~X~~	
1760c. 60µl	Inf548-7 a-2	HEU-lo ♀ TE8K	(3)	<8.26>			1	6.37	10.5	
2010c. 60µl	a-1	TE8J	(10)	<8.56>			1	6.09	10.1	
860c. 40µl	Inf315-9 a-1	HEU-hi ♂ SNK8	(4)	<4.19>			1	6.27	10.0	
020c. 40µl	a-2	SNK9	(8)	<10.11>			1	8.91	14.0	
730c. 40µl	ND050	Adult ♂	(7)	20E6 <11.03>	1/13/23		1	8.89	12.7	
100c. 4[illegible]	ND006	Adult ♀	(6)	15E6 <7.24>	5/12/23		1	13.5	16.9	

Start @ 9:26am 9:52 am

spin @ 49am 10:00 10:36

DNAse @ 10:10 10:13 10:54 10:20

11:15 am stain for count

11:30 am count

12:30 aliquot & resuspend cell

Incubation @ 12:56 → 6:56 pm

Reagents & Abs prepped @ 4:12 pm

→ sc spin @ 6:58 pm

L/D @ 7:19pm

hot SC's @ 7:25 → 7:55 spin @ 7:56

cold SC's @ 7:34 → 8:04 spin @ 8:08

spin @ 7:40 pm

<8:46 tets prepped>

Hot @ 8:54 pm → 8:24pm spin @ 8:27pm

SC Fix Perm 8:23 → :33 → :43 → 1st wash @ 8:47pm

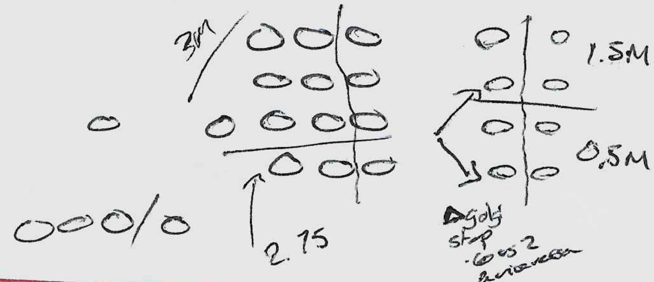
2nd wash @ 9:00pm

Tets @ 8:42pm →

Va Abs @ 8:52pm → 9:22 pm spin @ 9:27pm

9:16 Intrac SC's → 9:56

→ spin @ 9:58pm



9:41 pm Cold → 10:11 pm

RBC lyse @ 10:14pm

spin @ 10:20pm

10:31 pm → 41 → 51 pm

10:54pm first wash ✓

11:05pm 2nd wash ✓

11:17 pm → 11:52 Intracellular

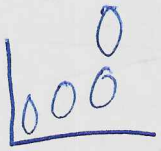
Done @ 00:07am ✓

Qc Aurora @ 2:48 pm (500 fixed)
2:54 pm sc start

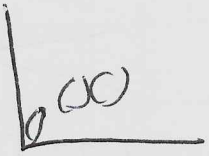
The APC CD3 PMA is probably @ AF647
1) alternative for CD16 unmixing on PMA.
weird mix?

Coffee @ 3:55 pm → 4:11 pm

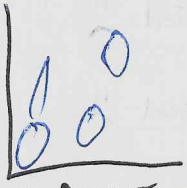
Brbe



Inf028
24K/31K
↑ 70/1



Inf548
29M 6M
Done @ 5:06



Inf315 *detctr 1 + cord unstained
25K/3.8M

i bin by cosine %?
vs reference?

Data Visualization

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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
INF028	11.96	2	5.98	0.50	0.46	0.42	0.38	0.33	0.29	0.25	0.21	0.17	0.13	0.08
INF548	12.46	2	6.23	0.48	0.44	0.40	0.36	0.32	0.28	0.24	0.20	0.16	0.12	0.08
INF315	15.18	2	7.59	0.40	0.36	0.33	0.30	0.26	0.23	~~0.20~~	0.16	0.13	0.10	0.07
INF000	2	2	1.00	3.00	2.75	2.50	2.25	2.00	1.75	1.50	1.25	1.00	0.75	0.50
ND050	8.89	1	8.89	0.34	0.31	0.28	0.25	0.22	0.20	0.17	0.14	0.11	0.08	0.06
ND006	13.5	1	13.50	0.22	0.20	0.19	0.17	0.15	0.13	0.11	0.09	0.07	0.06	0.04

1200 ×2 = 24 | 4 2µl ×2 = 8µl
100µl 1.83 ‖
110µl 2.00 ‖
110µl .dµl ‖

	Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
R10	INF028	0.50	0.456	0.415	0.37	0.332	0.290	0.25	0.207	0.166	0.12	0.083
	INF548	0.52	0.475	0.432	0.39	0.345	0.302	0.26	0.215	0.172	0.13	0.087
	INF315	0.60	0.554	0.504	0.45	0.402	0.352	~~0.30~~	0.251	0.201	0.15	0.101
	INF000	-2.00	-1.834	-1.667	-1.50	-1.334	-1.167	-1.00	-0.834	-0.667	-0.50	-0.333
	ND050	0.66	0.607	0.552	0.50	0.441	0.386	0.33	0.275	0.221	0.17	0.111
	ND006	0.78	0.712	0.648	0.58	0.518	0.453	0.39	0.323	0.259	0.19	0.130

	Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
PMA	INF028	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF548	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF315	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF000	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

	Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M
CD107a	INF028	6	5.5	5	4.5	4	3.5	3
	INF548	6	5.5	5	4.5	4	3.5	3
	INF315	6	5.5	5	4.5	4	3.5	3
	INF000	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	6.27	6.27	
1	8.91	8.91	7.59
		15.18	

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#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	13	L/D 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @37C	13	ColdStain 30min @4C	13	RBC Lyse, then Fix/Perm	Spiked 40 min @RT	13
1	UV2				BUV395	CD62L	SK11								1.2	15.6			
					AF	AF-UV6													
2	UV7				BUV496	CD8	RPA-T8												
3	UV9				BUV563	CD69	FN50								0.7	9.1			
4	UV10				BUV615	CCR4	1G1								0.5	6.5		1	13.0
5	UV11				BUV661	Vδ2	B6						2.0	26					
6	UV14				BUV737	CXCR3	1C6/CXCR3								0.7	9.1		0.1	1.3
7	UV16														1.3	16.9			
8	V1				BUV805	CD4	SK3						1.5	19.5				0.5	6.5
9	V3				BV421	CD127	A019D5						1.5	19.5					
	V3				Pacific Blue	CD14	M5E2								2.0	26.0			
10	V5				Pacific Blue	CD19	SJ25C1								2.0	26.0			
11	V7				BV480	CD161	HP-3G10						2	26					
					BV510	CD45RA	HI100								0.7	9.1			
12	V10				BV605	CD56	5.1H11						1.0	13				0.1	1.3
13	V11				BV650	CCR7	G043H7						1	13				0.7	9.1
14	V13				BV711	CD7	M-T701						1	13					
15	V14				BV750	IFNγ	B27											1.5	19.5
16	V15				BV786	CCR6	11A9						1.5	19.5					
17	B2				FITC/AF488	Va24/hCD1d	6B11					<:10/1.5>			1.2	15.6		0.1	1.3
18	B3				Spark blue 550	CD3	SK7						1.5	19.5				2.5	32.5
19					PerCP-Cy5.5	CD26	BA5b											0.5	6.5
20	B4				PE	NKG2D	1D11											1.5	19.5
21	B6				PE-Dazzle594	TNFa	MAB11						1.2	15.6				0.5	6.5
22	B8				PE-Cy5	CD25	M-A251								1.5	19.5		0.5	6.5
23	B13				PE-vio770	PD1	PD1.3.1.3								0.7	9.1			
24	R1				APC	CD16	3G8												
25	R2				AlexaFluor647	Va7.2/hMR1	3C10					<2:10/1.2>							
26	R4				APC-R700	CD107a	H4A3		6.0	78									
27	R6				Zombie NIR	L/D	N/A				<1:2500>								
28	R7				APC/Fire 750	CD27	O323						2	26					
29	R8				APC/Fire 810	CD38	HIT2						1.6	20.8					
							Antibody Total		6.0	78	Antibody Total		17.8	231	12.5	162.5		9.5	123.5
And UNSTAINED CONTROLS !!!							R10 Media		14.5	188.5	Brilliant Stain		50	650	50.0	650		11	143
							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)

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	13		
0.8	10.4	12.5	PBS
	4.16	5	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	13	104	3.9	3.9	52	0.39
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		179	6.9	12.9	154	1.39
		161.1			138.6	

FBS	89.5
PBS	89.5

PFA	0.139
PBS	1.251

Perm	15.4
Water	139

RBC	0.69
Water	6.21

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				B2	FITC/AF488	[55]	Vα24/hCD1d							
542	UV8				V7	BV510	[50]	CD45RA	B3	SparkBlue 550	[50]	CD3							
582	UV9	BUV563	[55]	CD69	V8	BV570			B4				YG1	PE	[50]	NKG2D				
598					V9				B5											
613	UV10	BUV615	[45]	CCR4	V10	BV605	[50]	CD56	B6				YG3	PE-Dazzle594	[60]	TNFα				
664	UV11	BUV661	[50]	Vδ2	V11	BV650	[50]	CCR7	B7								R1	APC	[50]	CD16
679									B8				YG5	PE-Cy5	[40]	CD25	R2	AF647	[50]	Vα7.2/hMR1
697	UV12				V12				B9	PerCP-Cy5.5	[45]	CD26					R3			
717	UV13				V13	BV711	[55]	CD7	B10								R4	APC-R700	[50]	CD107a
738									B11								R5			
750	UV14	BUV737	[40]	CXCR3	V14	BV750	[50]	IFNγ												
760									B12								R6	Zombie NIR	[55]	Viability
783	UV15				V15	BV786	[50]	CCR6	B13				YG9	Pe-Vio770	[50]	PD1	R7	APC-Fire 750	[45]	CD27
812	UV16	BUV805	[50]	CD4	V16				B14								R8	APC-Fire 810	[50]	CD38