May 30th, 2023

2023_ILT_04

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
2000c. 65µl Inf070-? a-5	HU ♀ NWICC	Box 3AC1	~5 <5.37 (5)			0.75	12.8	18.0	9.6
1850c. 65µl a-6	RWRD		<6.87 (9)		open, LN2 inside, thaw issue	0.75	10.6	15.5	7.95
1800c. 65µl Inf374-4 a-9	HEU-lo ♀ SVS6	Box9B H8	~7.5 <6.67 (4)			0.75	9.5	13.6	7.12
1780c. 65µl a-10	SVS1		<6.17 (7)			0.75	8.1	12.6	6.08
65µl Inf287-7 a-4	HEU-lo ♀ SLZ1	Box7B B10	~5 (3)			0.75			4.70 ↑
1460c. 65µl a-7	SLZ2		<8.17 (8)		loose tape! thaw issue	0.75	6.3	11.7	4.70
1950c. 65µl Inf191-1 a-5	HEU-hi ♀ SLAN	Box6BC7	~10 (6)		Vapory, loose lid?	1	7.1	13.2	
850c. 40µl ND050	Adult		15EC <11.47 (2)	1/13/23		1	8.5	12.2	
830c. 40µl ND006	Norm		15EC <8.27 (1)	1/20/23		1	11.8	14.7	

10:18 am Adult Thaw 10:41 DNAse adult
10:31 am cool 10:47 Spin 11:04 DNAse
10:51 am Load alignment 287 11:14 DNAse
11:39 am stain for count
12:09 pm count done
12:29 aliquots
1:08 pm RIO's
1:24 ND006

n=17 n=10

2.5M

1:43pm Cells into Incubator → 7:43pm

★ Note Inf191 vs PMA ↑ pmadose vs ND050 vs PMA
Maybe up to x2?. ~~Not necessary~~
~~@ this point~~, but note if aberrant ↑ 1:14a raised 830µl added +5µl CODA to restore PMA/ml balance, at cost cells/µL conc → 3.0 1.5M

5:38 most Abs & reagents prepped
Exhaustive search for BV430 CD16, CD2, CD41
6:03 everything - zombie NIR prepped //

7:51pm Spin @
WO @ 8:09 - Spin @ 8:29pm
20:24 sc spin
Hot samples @ 8:47 - 9:17 pm Spin @ 9:21

n=36 sc's

Zombie sc's @ 8:52 → 9:07pm
hot sc's @ 8:59 → 9:29
Cold sc's @ 9:08 → 9:38
9:32 Tets prepped
Tets @ 9:48 → 10:28 pm Spin @ 9:42
Abs @ 10:00 → 10:30pm
Sc FixPerm @ 9:56 → 06 → 16
sc First spin @ 10:22
Cold samples @ 22:49 → 23:19
Sc's intrac @ 10:58 → 16:38pm
Sc's done @ 11:06pm
Rbc lysc @ 11:26 pm
Spin @ 11:34pm Mord specimens

Sample Fix Perm @ 11:48 → 58 → 00:08

SCS done @ 11:50 pm w/ 20 μl of 0.4% PFA

→ 1st wash @ 12:12 am

2nd wash @ 12:24 am

Intrac @ 12:37 am → 1:17 am

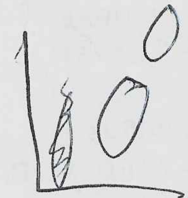
1:19 am final spin

Done @ 1:33 am, placed in 30 μl

In cell pop veritas

¿ σ of a marker expression?

Coloring w/in the lines

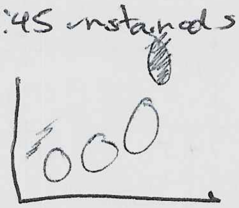


NW050 ~~55 μl~~ 37 μl

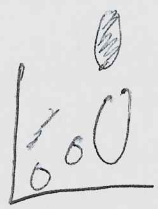
Start @ 8:30 pm <35 μl> actually a ctrl well this time.

200,000

9:45 instarted s



Inf010 ctrl



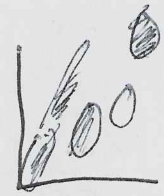
Inf374

4900/92 55 μl

PMA 4100/68 45 μl

6200/147 45 μl

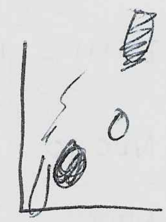
6100/140 43 μl



Inf287 43 μl

(poorer shape) 51 μl

↑ evidence extra AF



Inf191

35 μl ~~54 μl~~ 41 μl

Very few alive in PMA

10:07 15000/943 48 μl 60 μl

2.5 m cells / tube

events

★ ¿How communicate effectively to peers, SRLs, etc not in same frame/thought?★

★ Inf374 ~~82 μl~~ tet → hiccup clump 7 μl. ★

★ How much variation per calling is acceptable? ★

287 2500/3900

Done @ 11:00 pm

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
INF070	17.55	1.5	11.70	0.26	0.24	0.21	0.19	0.17	0.15	0.13	0.11	0.09	0.06	0.04
INF374	13.2	1.5	8.80	0.34	0.31	0.28	0.26	0.23	0.20	0.17	0.14	0.11	0.09	0.06
INF287	9.45	1.5	6.30	0.48	0.44	0.40	0.36	0.32	0.28	0.24	0.20	0.16	0.12	0.08
INF191	7.7	1	7.70	0.39	0.36	0.32	0.29	0.26	0.23	0.19	0.16	0.13	0.10	0.06
ND050	8.5	1	8.50	0.35	0.32	0.29	0.26	0.24	0.21	0.18	0.15	0.12	0.09	0.06
ND006	11.8	1	11.80	0.25	0.23	0.21	0.19	0.17	0.15	0.13	0.11	0.08	0.06	0.04

R10

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF070	0.74	0.681	0.619	0.56	0.495	0.433	0.37	0.309	0.248	0.19	0.124
INF374	0.66	0.604	0.549	0.49	0.439	0.384	0.33	0.274	0.219	0.16	0.110
INF287	0.52	0.479	0.436	0.39	0.349	0.305	0.26	0.218	0.174	0.13	0.088
INF191	0.61	0.559	0.508	0.46	0.406	0.356	0.31	0.254	0.203	0.15	0.102
ND050	0.65	0.592	0.539	0.49	0.431	0.377	0.32	0.269	0.215	0.16	0.108
ND006	0.75	0.683	0.621	0.56	0.497	0.435	0.37	0.310	0.248	0.19	0.125

PMA

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF070	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF374	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF287	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF191	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
INF070	6	5.5	5	4.5	4	3.5	3
INF374	6	5.5	5	4.5	4	3.5	3
INF287	6	5.5	5	4.5	4	3.5	3
INF191	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
0.75	6.3	4.725	
0.75	6.3	4.725	**4.725**
		9.45	

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ran out ○

#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	17	L/D 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @37C	17	ColdStain 30min @4C	17	RBC Lyse, then Fix/Perm	Spiked 40 min @RT	17
1	UV2				BUV395	CD62L	SK11								1.2	20.4			
2	UV7				AF	AF-UV6													
3	UV9				BUV496	CD8	RPA-T8								0.7	11.9			
4	UV10				BUV563	CD69	FN50								0.5	8.5		1	17.0
5	UV11				BUV615	CCR4	1G1						2.0	34					
6	UV14				BUV661	Vδ2	B6								0.7	11.9		0.1	1.7
7	UV16				BUV737	CXCR3	1C6/CXCR3								1.3	22.1			
8	V1				BUV805	CD4	SK3						1.5	25.5				0.5	8.5
9	V3				BV421	CD127	A019D5						1.5	25.5					
	V3				Pacific Blue	CD14	M5E2								2.0	34.0			
10	V5				Pacific Blue	CD19	SJ25C1								2.0	34.0			
11	V7				BV480	CD161	HP-3G10						2	34					
					BV510	CD45RA	HI100								0.7	11.9			
12	V10				BV605	CD56	5.1H11												
13	V11				BV650	CCR7	G043H7						1.0	17				0.1	1.7
14	V13				BV711	CD7	M-T701						1	17				0.7	11.9
15	V14				BV750	IFNγ	B27						1	17					
16	V15				BV786	CCR6	11A9											1.5	25.5
17	B2				FITC/AF488	Va24/hCD1d	6B11						1.5	25.5					
18	B3				Spark blue 550	CD3	SK7					<:10/1.5>			1.2	20.4		0.1	1.7
19					PerCP-Cy5.5	CD26	BA5b											2.5	42.5
20	B4				PE	NKG2D	1D11						1.5	25.5				0.5	8.5
21	B6				PE-DazzleS94	TNFa	MAB11											1.5	25.5
22	B8				PE-Cy5	CD25	M-A251						1.2	20.4				0.5	8.5
23	B13				PE-vio770	PD1	PD1.3.1.3								1.5	25.5		0.5	8.5
24	R1				APC	CD16	3G8								0.7	11.9			
25	R2				AlexaFluor647	Va7.2/hMR1	3C10					<2:10/1.2>							
26	R4				APC-R700	CD107a	H4A3		6.0	102									
27	R6				Zombie NIR	L/D	N/A				<1:2500>								
28	R7				APC/Fire 750	CD27	O323												
29	R8				APC/Fire 810	CD38	HIT2						2	34					
													1.6	27.2					
						Antibody Total			6.0	102	Antibody Total		17.8	303	12.5	212.5		9.5	161.5
And UNSTAINED CONTROLS !!!						R10 Media			14.5	246.5	Brilliant Stain		50	850	50.0	850		11	187
						Pippette draw volume /sample			19.5		Pippette draw volume /sample		65		59.5			19.5	

last vial?

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			10
FITC	Va24Ja18	1.5	15
AF647	Va7	1.2	12
	PBS	17.8	178
Pippette draw volume/sample:		19.5	

Tetramer Mix			5
AF488	hCD1d PBS-57	0.5	3
AF647	hMR1 5-OP-RU	2	12

Tetramer Control Mix			2
AF488	hCD1d Unloaded	0.5	1.2
AF647	hMR1 6-FP	2	4.8

Zombie NIR	19		
0.8	15.2	17.5	PBS
	6.08	7	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	17	136	5.1	5.1	68	0.765
Number Unstained	10	80	3	2	20	0.25
Number Surface SCs (34)	30	60	0	6	60	0.75
Number Intracellular SCs	6	12	0	1.2	24	0.15
Total		288	8.1	14.3	172	1.915

FBS	144
PBS	144

PFA	0.1915
PBS	1.7235

Perm	17.2
Water	155

RBC	0.81
Water	7.29

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									
613	UV10	BUV615	[45] CCR4	V10	BV605	[50] CD56	B5			YG1	PE	[50] NKG2D				
664	UV11	BUV661	[50] Vδ2	V11	BV650	[40] CCR7	B6									
679							B7			YG3	PE-Dazzle594	[60] TNFα				
697	UV12						B8						R1	APC	[55]	CD16
717	UV13			V12			B9	PerCP-Cy5.5	[45] CD26	YG5	PE-Cy5	[40] CD25	R2	AF647	[50]	Vα7.2/hMR1
738				V13	BV711	[55] CD7	B10						R3			
750	UV14	BUV737	[40] CXCR3				B11						R4	APC-R700	[55]	CD107a
760				V14	BV750	[50] IFNγ							R5			
783	UV15						B12									
812	UV16	BUV805	[50] CD4	V15	BV786	[50] CCR6	B13			YG9	Pe-Vio770	[50] PD1	R6	Zombie NIR	[65]	Viability
				V16			B14						R7	APC-Fire 750	[45]	CD27
													R8	APC-Fire 810	[50]	CD38

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