June 12th, 2023

2023_ILT_08

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
Inf662-7 a-3	HU ♀ TNGH	(8)	(~8m) <12.15?			1.5	5.43	6.8	
a-4	TNGS	(7)	<12.19?			1.5	6.11	7.73	
Inf356-4 a-2	HEU-lo♂ SU3V	(3)	<11.6?			1	7.27	11.12	
a-1	SU3V	(a)	<9.6?			1	8.21	12.1	
Inf479-0 a-1	HEU-lo ♀ T32H	(4)	<8.29?			2	8.04	10.8	
a-2	TJRM	(10)	<6.7?			2	10.3	13.7	
Inf [redacted] 387-8 a-2	HEU-hi ♀ SVW1	(2)	[redacted] <22.8?			1	3.91	5.19	
a-1	SVW0	(6)	<13.15?			1	6.74	8.79	
NDO50	Adult ♂	(5)	15E6 <10.9?	[redacted] 1/13/23		1	9.21	12.0	
NDO06	Adult ♀	(1)	15E6 <6.7?	5/12/13		1	14.80	17.5	

1720c. 8
1510c. 80/1
1060c. 60/1
1050c. 60/1
1250c. 80/1
1340c. 80/1
740c. 40/1
940c. 60/1
740c. 40/1
1040c. 40/1

Start @ 1:20 pm (thaw) (spin) (DNAse) (recount)
adult @ 1:25 pm @ 1:39 @ 1:54 @ 2:09
@ @ 1:53 @ 2:15 @
@ @ 2:10 @ 2:26 a

2.45pm stain for count
3:01 pm count
3:60 pm aliquot

Incubation @ 4:25 pm

9:53 reagent prep start

10:28 abs went flying... fuck... going be a long night.

Sample spin @ 10:36
L/D @ 10:52pm →
Abs prepped @ 11:04 pm spin @ 11:10
sc's aliquoted @ 11:19pm
spin @ 11:24pm

Hots @ 11:31 → 00:01
Sc L/D @ 11:39
Hot Sc's @ 11:47pm
Cold sc's @ 11:55pm

Tube @ 00:22 →
abs @ 32 → 1:02am
35
↑ NDO50 PMA might not have gotten full dose

0 0 0 | 0
0 0 0 | 0
0 0 0 0 | 0
0 0 0 | 0
0 0 0 | 0
0 0 0 | 0

66.3 / 1.98 150

0.53 / 0.66 ≤ 2 in 100

16 / 32 ≤2

6 / 12 ≤2

Sc's FixPerm @ 00:42 → 5E → 0E

1:12am combined spin

1:26am Colds → 5E

1:34am 2nd sc permwash spin

1:50am Intra Sc's. → 2:30 am

RBC lysis @ 1:58 am

Spin @ 2:06 am

Samples fix @ 2:19 → 2E → 3E

1st permwash @ 2:43 am

2nd @ 2:55 am

3:09 am Intracellular (ooof!!)

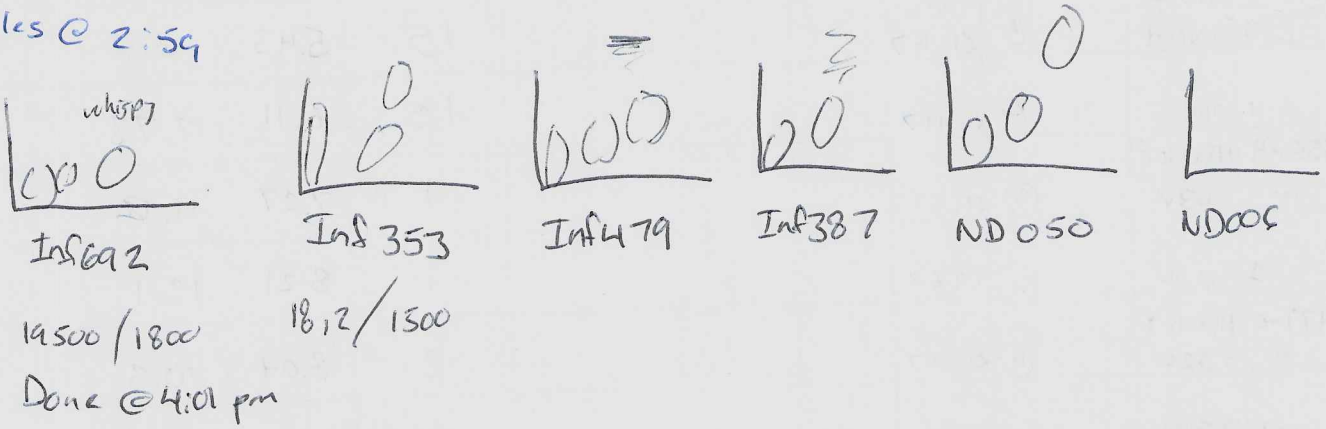
Final wash @ 3:49 am

Done @ 4:01 am

MarkOFF in log

running water @ 1:47 pm
UV's @ 1:49 pm (200 events, 5 µl left)
sds done @ 2:55 pm

Samples @ 2:59



19500/1800

18,12/1500

Done @ 4:01 pm

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334
-140
194

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
INF692	17.31	3	5.77	0.52	0.48	0.43	0.39	0.35	0.30	0.26	0.22	0.17	0.13	0.09
INF356	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
INF479	36.68	4	9.17	~~0.33~~ 400	0.30	0.27	0.25	0.22	0.19	0.16	0.14	~~0.11~~ 140	0.08	0.05
INF387	10.65	2	5.33	0.56	0.52	0.47	0.42	0.38	0.33	0.28	0.23	0.19	0.14	0.09
ND050	9.21	1	9.21	0.33	0.30	0.27	0.24	0.22	0.19	0.16	0.14	0.11	0.08	0.05
ND006	14.8	1	14.80	~~0.20~~ 250	0.19	0.17	0.15	0.14	0.12	0.10	0.08	0.07	0.05	0.03

	Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
R10	INF692	0.48	0.439	0.400	0.36	0.319	0.280	0.24	0.199	0.160	0.12	0.080
	INF356	0.61	0.561	0.510	0.46	0.408	0.357	0.31	0.255	0.204	0.15	0.102
	INF479	~~0.67~~ 600	0.616	0.560	0.50	0.448	0.392	0.34	0.280	~~0.224~~ 194	0.17	0.112
	INF387	0.44	0.400	0.364	0.33	0.290	0.254	0.22	0.181	0.145	0.11	0.073
	ND050	0.67	0.617	0.562	0.51	0.449	0.393	0.34	0.280	0.224	0.17	0.113
	ND006	~~0.80~~ 70	0.730	0.664	0.60	0.531	0.465	0.40	0.332	0.265	0.20	0.133

	Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
PMA	INF692	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF356	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF479	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF387	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	INF692	6	5.5	5	4.5	4	3.5	3
	INF356	6	5.5	5	4.5	4	3.5	3
	INF479	6	5.5	5	4.5	4	3.5	3
	INF387	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	3.91	3.91	
1	6.74	6.74	**5.325**
		10.65	

June 12th, 2023 ILT #8

#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	16	L/D 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @37C	16	ColdStain 30min @4C	16	RBC Lyse, then Fix/Perm	Spiked 40 min @RT	16
1	UV2				BUV395	CD62L	SK11								1.2	19.2			
2	UV7				AF	AF-UV6													
3	UV9				BUV496	CD8	RPA-T8												
4	UV10				BUV563	CD69	FN50								0.7	11.2			
5	UV11				BUV615	CCR4	1G1								0.5	8.0		1	16.0
6	UV14				BUV661	Vδ2	B6						2.0	32					
7	UV16				BUV737	CXCR3	1C6/CXCR3								0.7	11.2		0.1	1.6
8	V1				BUV805	CD4	SK3								1.3	20.8			
9	V3				BV421	CD127	A019D5						1.5	24				0.5	8.0
	V3				Pacific Blue	CD14	M5E2						1.5	24					
10	V5				Pacific Blue	CD19	SJ25C1								2.0	32.0			
11	V7				BV480	CD161	HP-3G10								2.0	32.0			
					BV510	CD45RA	HI100						2	32					
12	V10														0.7	11.2			
13	V11				BV605	CD56	5.1H11												
14	V13				BV650	CCR7	G043H7						1.0	16				0.1	1.6
15	V14				BV711	CD7	M-T701						1	16				0.7	11.2
16	V15				BV750	IFNγ	B27						1	16					
17	B2				BV786	CCR6	11A9											1.5	24.0
18	B3				FITC/AF488	Va24/hCD1d	6B11						1.5	24					
19					Spark blue 550	CD3	SK7					<:10/1.5>							
20	B4				PerCP-Cy5.5	CD26	BA5b								1.2	19.2		0.1	1.6
21	B6				PE	NKG2D	1D11											2.5	40.0
22	B8				PE-DazzleS94	TNFa	MAB11						1.5	24				0.5	8.0
23	B13				PE-Cy5	CD25	M-A251											1.5	24.0
24	R1				PE-vio770	PD1	PD1.3.1.3						1.2	19.2				0.5	8.0
25	R2				APC	CD16	3G8								1.5	24.0		0.5	8.0
26	R4				AlexaFluor647	Va7.2/hMR1	3C10								0.7	11.2			
27	R6				APC-R700	CD107a	H4A3		6.0	96		<2:10/1.2>							
28	R7				Zombie NIR	L/D	N/A				<1:2500>								
29	R8				APC/Fire 750	CD27	O323						2	32					
					APC/Fire 810	CD38	HIT2						1.6	25.6					
							Antibody Total		6.0	96	Antibody Total		17.8	285	12.5	200.0		9.5	152
And UNSTAINED CONTROLS !!!							R10 Media		14.5	232	Brilliant Stain		50	800	50.0	800		11	176
							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			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			1
AF488	hCD1d Unloaded	0.5	0.6
AF647	hMR1 6-FP	2	2.4

Zombie NIR	18		
0.8	14.4	15	PBS
	5.76	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	16	128	4.8	4.8	64	0.48
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		203	7.8	13.8	166	1.48
		182.7			149.4	

FBS	101.5
PBS	101.5

PFA	0.148
PBS	1.332

Perm	16.6
Water	149

RBC	0.78
Water	7.02

ILT & NK SFC Panel

Spectrum	UV			Violet			Blue			Yellow-Green			Red		
373	UV1														
388	UV2	BUV395	[30] 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	[30] CD45RA	B2	FITC/AF488	[50] 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	[30] CCR7	B6			YG3	PE-Dazzle594	[60] TNFα			
679							B7						R1	APC	[30] CD16
697	UV12			V12			B8			YG5	PE-Cy5	[50] CD25	R2	AF647	[30] 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	[30] 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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