~400p/l cDOT

July 7th, 2023

2023_ILT_13

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
Inf557-7 a-5	HU♂ TES4	(8)	<??>			1	4.72	9.06	
Inf557-7 a-4	TES3								
Inf599-3 a-2	HU♂ THSK	(1)	<7.59>			0.5	8.71 /	14.6 /	
a-3	THSL	(11)	<7.71>			0.5	8.43 /	14.2 /	
Inf173-1 a-1	HEU-lo ♀ S98L	(4)	<8.02>			1	6.99	12.7	
a-2	S98M	(13)	7.82			1	7.78	12.6	
Inf274-3 a-1	HEU-hi ♀ SG2N	(2)	<9.38>			1	9.16	12.2	
a-2	SG2P	(12)	<9.07>			1	9.77	13.4	
Inf342-9 a-1	HEU-hi ♀ SG3N	(3)	<7.21>			1	4.26	6.36	
a-2	SG3P	(10)	<6.63>			1	4.75	7.00	
Inf269-7 a-2	Heu-hi ♀ SGP2	(5)	<8.35>			0.5	11.1 /	16.1 /	
a-1	SGPY	(9)	<8.82>			0.5	10.7 /	16.1 /	
ND050		(7)	20E6 <10.41>	1/13/23		1	9.32	12.3	
ND006		(6)	15E6 <6.87>	5/12/23		1	14.3	16.9	

1970c. 70p
1890c. 50p/l
1640c. 50p/l
2210c. 50p/l
1530c. 50p/l
1240c. 40p/l
980c. 40p/l
1120c. 70p/l
1600c. 70p/l
250c. 40p/l
250c. 40p/l
720c. 40p/l
070c. 40p/l

Start @ 10:13 am → spin 10:35 | 11:13 | 11:19
only scanned (1)557, other Δ samples

second set spin @ 11:10 am 11:30
11:50 am stain for count
Count @ 12:05 pm → 12:15 pm
~~Δ~~ 2:34 Inf557 aliquot #2 spin~~*~~ done 14:46
1:41 aliquot & resus.

2:42 pm Incubation start → 8:42 pm

AB prep @ 7:34 pm → 8:12
reagents prepped @ 8:27
8:45 pm sc spin
8:55 pm sample spin
4:08 pm hot scs → 9:38 spin @ 9:42
L/Q @ 9:15 pm → 9:30

Cold scs @ 9:27 pm → ~~9~~ 5:7 pm
L/O spin @ 9:34 pm → spin @ 10:03
Hot samples @ 9:59 pm → 10:29
10:09 pm tets prepped → spin @ 10:40
10:17 pm → 27 → 37 scs fix p.m.
→ spin @ 34
tets @ 10:57 → 11:36 pm
Vols 11:06 extra @ 11:12 → 52 pm

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→ 36pm samples → spin@45pm
11:52pm - JC's intern

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Cold stain samples @ 12:05pm → 12:35am

12:08am sets done

Rbclyse @ 12:39 am → 12:42 am

↳ spin @ 12:47 am

Samples FixPerm @ 1:02 → 12 → 22

1st wash @ 1:27pm

2nd wash @ 1:43 pm

Intracellular 1:58 pm → 2:38 am

sc's & unstaineds → 15µl

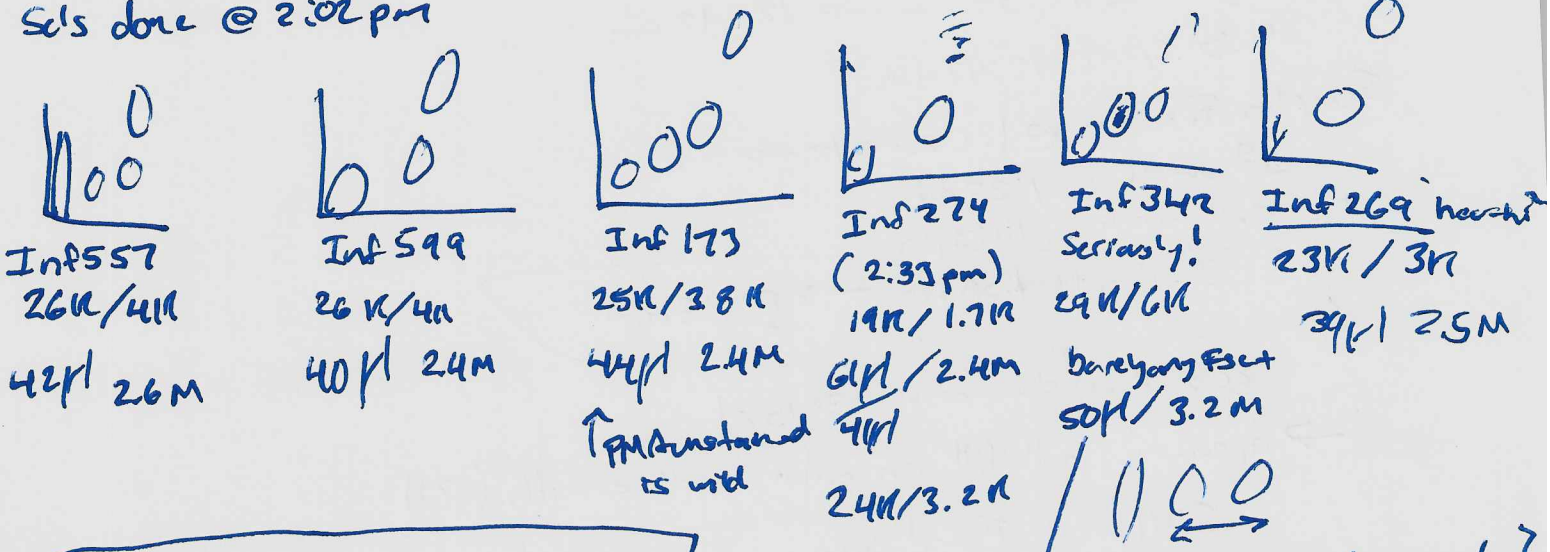
→ appears will fill an entire rack exactly (72)

2:41 pm Final spin

Stashed @ 2:55pm (samples 25µl)

→ Lids not clicking ½ tubes

Acquisition QC @ 1:03 pm
SC start @ 1:10 pm
Inf274 looks clean per SC's ~22k/l
TNFa ↔ NR6ZD (fixed ✓)
SC's done @ 2:02 pm



Inf557
26K/41K
42k/l 2.6M

Inf599
26K/4M
40k/l 2.4M

Inf173
25K/38K
44k/l 2.4M
↑ PMA unstained is wild

Inf274
(2:33 pm)
19K/1.7K
6Gk/l / 2.4M
4k/l
24K/3.2K

Inf342
Serial!
29K/6K
Barely any Fact
50k/l / 3.2M

Inf269 hearsh?
23K / 3K
39k/l 2.5M

Inf342 poor quality !!
HEU-hi

At least the controls ≠ PMA ehh...

? PMA ex change rates?
Quantitative/modeling

Something odd w/ pma/ctrl unstained toward experimental Δ pma time?

Done @ 3:12 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
INF557	9.44	2	4.72	0.64	0.58	0.53	0.48	0.42	0.37	0.32	0.26	0.21	0.16	0.11
INF599	8.57	1	8.57	0.35	0.32	0.29	0.26	0.23	0.20	0.18	0.15	0.12	0.09	0.06
INF173	14.77	2	7.39	0.41	0.37	0.34	0.30	0.27	0.24	0.20	0.17	0.14	0.10	0.07
INF274	18.93	2	9.47	0.32	0.29	0.26	0.24	0.21	0.18	0.16	0.13	0.11	0.08	0.05
INF342	9.01	2	4.51	0.67	0.61	0.55	0.50	0.44	0.39	0.33	0.28	0.22	0.17	0.11
INF269	10.9	1	10.90	0.28	0.25	0.23	0.21	0.18	0.16	0.14	0.11	0.09	0.07	0.05
ND050	9.32	1	9.32	0.32	0.30	0.27	0.24	0.21	0.19	0.16	0.13	0.11	0.08	0.05
ND006	14.3	1	14.30	0.21	0.19	0.17	0.16	0.14	0.12	0.10	0.09	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	INF557	0.36	0.333	0.303	0.27	0.242	0.212	0.18	0.151	0.121	0.09	0.061
	INF599	0.65	0.595	0.541	0.49	0.433	0.379	0.32	0.270	0.216	0.16	0.109
	INF173	0.59	0.544	0.494	0.45	0.395	0.346	0.30	0.247	0.198	0.15	0.099
	INF274	0.68	0.625	0.569	0.51	0.455	0.398	0.34	0.284	0.227	0.17	0.114
	INF342	0.33	0.306	0.278	0.25	0.222	0.195	0.17	0.139	0.111	0.08	0.056
	INF269	0.72	0.664	0.604	0.54	0.483	0.422	0.36	0.301	0.241	0.18	0.121
	ND050	0.68	0.621	0.565	0.51	0.451	0.395	0.34	0.282	0.226	0.17	0.113
	ND006	0.79	0.724	0.658	0.59	0.526	0.461	0.40	0.329	0.263	0.20	0.132

	Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
PMA	INF557	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF599	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF173	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF274	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF342	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF269	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	INF557	6	5.5	5	4.5	4	3.5	3
	INF599	6	5.5	5	4.5	4	3.5	3
	INF173	6	5.5	5	4.5	4	3.5	3
	INF274	6	5.5	5	4.5	4	3.5	3
	INF342	6	5.5	5	4.5	4	3.5	3
	INF269	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
0.5	11.1	5.55	
0.5	10.7	5.35	**5.45**
		10.9	

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#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	22	L/D 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @37C	22	ColdStain 30min @4C	22	RBC Lyse, then Fix/Perm	Spiked 40 min @RT	22
1	UV2				BUV395	CD62L	SK11								1.2	26.4			
2	UV7				AF	AF-UV6													
3	UV9				BUV496	CD8	RPA-T8								0.7	15.4			
4	UV10				BUV563	CD69	FN50								0.5	11.0		1	22.0
5	UV11				BUV615	CCR4	1G1						2.0	44					
6	UV14				BUV661	Vδ2	B6								0.7	15.4		0.1	2.2
7	UV16				BUV737	CXCR3	1C6/CXCR3								1.3	28.6			
8	V1				BUV805	CD4	SK3												
9	V3				BV421	CD127	A019D5						1.5	33				0.5	11.0
	V4				Pacific Blue	CD14	M5E2						1.5	33					
10	V5				Pacific Blue	CD19	SJ25C1								2.0	44.0			
11	V7				BV480	CD161	HP-3G10								2.0	44.0			
					BV510	CD45RA	HI100						2	44					
12	V10														0.7	15.4			
13	V11				BV605	CD56	5.1H11												
14	V13				BV650	CCR7	G043H7						1.0	22	old			0.1	2.2
15	V14				BV711	CD7	M-T701						1	22				0.7	15.4
16	V15				BV750	IFNγ	B27						1	22					
17	B2				BV786	CCR6	11A9											1.5	33.0
18	B3				FITC/AF488	Va24/hCD1d	6B11						1.5	33					
19					Spark blue 550	CD3	SK7					<:10/1.5>							
20	B4				PerCP-Cy5.5	CD26	BA5b								1.2	26.4		0.1	2.2
21	B6				PE	NKG2D	1D11											2.5	55.0
22	B8				PE-Dazzle594	TNFa	MAB11						1.5	33				0.5	11.0
23	B13				PE-Cy5	CD25	M-A251											1.5	33.0
24	R1				PE-vio770	PD1	PD1.3.1.3						1.2	26.4				0.5	11.0
25	R2				APC	CD16	3G8								1.5	33.0		0.5	11.0
26	R4				AlexaFluor647	Va7.2/hMR1	3C10								0.7	15.4			
27	R6				APC-R700	CD107a	H4A3		6.0	132		<2:10/1.2>							
28	R7				Zombie NIR	L/D	N/A				<1:2500>								
29	R8				APC/Fire 750	CD27	O323						2	44					
					APC/Fire 810	CD38	HIT2						1.6	35.2					
						Antibody Total			6.0	132	Antibody Total		17.8	392	12.5	275.0		9.5	209
And UNSTAINED CONTROLS !!!						R10 Media			14.5	319	Brilliant Stain		50	1100	50.0	1100		11	242
						Pippette draw volume /sample			19.5		Pippette draw volume /sample		65		59.5			19.5	

new

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			14
FITC	Va24Ja18	1.5	21
AF647	Va7	1.2	16.8
	PBS	17.8	249.2
Pippette draw volume/sample:		19.5	

Tetramer Mix			7
AF488	hCD1d PBS-57	0.5	4.2
AF647	hMR1 5-OP-RU	2	16.8

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

Zombie NIR	22		
0.8	17.6	20	PBS
	7.04	8	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	22	176	6.6	6.6	88	0.66
Number Unstained	14	56	4.2	2.8	28	0.42
Number Surface SCs (29)	30	30	0	6	60	0.6
Number Intracellular SCs	6	6	0	1.2	24	0.12
Total		268	10.8	16.6	200	1.8
		241.2			180	

FBS	134
PBS	134

PFA	0.18
PBS	1.62

Perm	20
Water	180

RBC	1.08
Water	9.72

ILT & NK SFC Panel

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

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