June 27th, 2023

2023_ILT_09

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
Inf250-7 a-5	Hu ? SFM6	(12)	<9.697		Bloody	1	5.96	10.5	
a-6	SFM7	(8)	<9.097			1	5.98	10.6	
Inf169-6 a-2	SQ0V HEU-lo ?	(2)	<21.787		Bloody-est	1	3.14	5.82	
a-1	SQ0U	(7)	<14.747			1	4.43	7.58	
Inf101-2 a-4	HEU-hi RYBH	(3)	<15.047			1	4.81	7.04	
a-3	RYB6	(10)	<15.47			1	5.25	7.86	
Inf320-5 a-1	HEU-hi ? SNUY	(1)	<9.37			1	7.16	8.76	
a-2	SNUZ	(9)	<7.97			1	8.04	9.57	
ND050		(5)	ISE6.7 <11.127	1/13/23		1	9.21	12.4	
ND006		(6)	ISE6 <6.657	5/12/23		1	14.9	17.4	

1960c. 70µ
1960c. 80µl
1950c. 80µl
2100c. 80µl
1300c. 40µl
1400c. 60µl
1540c. 60µl
1510c. 60µl
960c. 40µl
1430c. 40µl

1st thaw @ 9:23 spin @ 9:39 DNAse @ 9:58 resus @ 10:11
adult thaw 9:41 spin @ 9:49 DNAse @ 10:04 resus @ 10:11
2nd thaw @ 10:27 spin @ DNAse @ 10:45 resus @

3.79
7.57
11.86

11:02 am stain count

Count @ 11:19 am 11:42 am 3rd Aliquot of Inf169 a-3 SQ0W

12:12 aliquot starts:
→ 31

~~Incubation @ 1:00 pm~~

<Ab prep @ 6:30 pm >

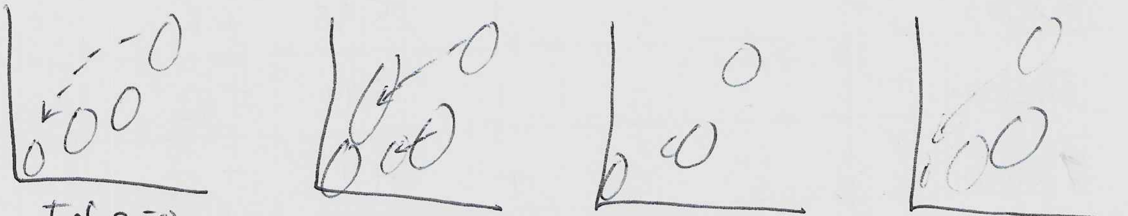
7:09 spin samples
7:25 L/D → 7:40 pm
7:32 sc spin
L/D sc @ 7:49 → 8:04 pm
Hots @ 8:03 pm → 8:33 pm
Hot sc's 8:11 → 8:41 pm
Cold sc's @ 8:21 pm → 8:51 pm
Tet's prepped @ 8:29 pm
8:50 pm Tets → 9:30
9:04 Abs → 9:34 pm
UPS 200 → 20µl pipette 9:00 → 9:13 pm (as they get 6x 3/2 = 9x)

9:10 pm sc Fix Perm → 20 → 30
Combined spin @ 9:37 pm
9:50 cold samples → 10:20 pm
9:58 2nd Perm Wash
10:12 pm Intra sc's → 10:52 pm → spin @ 10:53
10:24 pm RBC lysis spin @ 10:27 pm
10:40 pm → 50 → 11:00 pm Fix Perm samples
2nd perm wash @ 11:16 pm none
11:29 Intrac for samples (1 little left for tet ctrl well)
→ 12:09 am Final spin @ 12:10
Done @ 12:22 AM

Acquisition start @ 4:03 pm

ND050 & PMA
¿ ~~Ctrl~~ unstained off Y6 - R8 ↑↑↑ signal?

Samples @ 5:32pm:



Inf 250	Inf 169	Inf 101	Inf 320	
☆Vactrl screened	☆Vactrl screen	15K/1K	15K/1K	Cunningham's law (internet forums)
	25K/3.3K			

Using ND050 ctrl/PMA as Unstained's given whatever happened w/ ND006's ↑Y6+UV (L86.16 w/ both Fitc/AF488 unit)

Antibodies reunmixed ☐ Tetramers reunmixed ☐

		Tetramer					
ND050	0.04	NRT	0.33			0.02	~~0.5~~ 1.05
	0.05		0.02			0.04	0.08
101	0.12		0.02	~~0.04~~	~~0.08~~	0.07	0.03
	0.02		0.01	~~0.03~~	~~0.13~~	0.03	0.11
250	0.06		0.04	~~0.03~~	~~0.10~~	0.03	0.08

heod ed comparable, the hMR1 OP-RU feeling.

June 27th, 2023

2023_ILT_09

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
INF250	11.94	2	5.97	0.50	0.46	0.42	0.38	0.34	0.29	0.25	0.21	0.17	0.13	0.08
INF169	11.36	3	3.79	0.79	0.73	0.66	0.59	0.53	0.46	0.40	0.33	0.26	0.20	0.13
INF101	10.06	2	5.03	0.60	0.55	0.50	0.45	0.40	0.35	0.30	0.25	0.20	0.15	0.10
INF320	15.2	2	7.60	0.39	0.36	0.33	0.30	0.26	0.23	0.20	0.16	0.13	0.10	0.07
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	14.9	1	14.90	0.20	0.18	0.17	0.15	0.13	0.12	0.10	0.08	0.07	0.05	0.03

2 x650 / 12 PMA

1600 / <2 / 32 ctrl

.66 x3 / 1.00

.33 <2 / .66 in 100 ish

	Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
R10	INF250	0.50	0.455	0.414	0.37	0.331	0.290	0.25	0.207	0.165 115	0.12	0.083
	INF169	0.21	0.190	0.173	0.16	0.138	0.121	0.10	0.086	0.069 19	0.05	0.035
	INF101	0.40	0.369	0.336	0.30	0.268	0.235	0.20	0.167	0.134	0.10	0.068 18
	INF320	0.61	0.554	0.504	0.45	0.403	0.353	0.30	0.252	0.201 151	0.15	0.101
	ND050	0.67	0.617	0.562	0.51	0.449	0.393	0.34	0.280	0.224	0.17	0.113 63
	ND006	0.80	0.731	0.665	0.60	0.532	0.466	0.40	0.332	0.266	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	INF250	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF169	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF101	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF320	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	INF250	6	5.5	5	4.5	4	3.5	3
	INF169	6	5.5	5	4.5	4	3.5	3
	INF101	6	5.5	5	4.5	4	3.5	3
	INF320	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	7.16	7.16	
1	8.04	8.04	**7.6**
		15.2	

June 27th, 2023 ILT #9

#	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								0.7	11.2			
4	UV10				BUV563	CD69	FN50								0.5	8.0		1	16.0
5	UV11				BUV615	CCR4	1G1						2.0	32					
6	UV14				BUV661	Vδ2	B6								0.7	11.2		0.1	1.6
7	UV16				BUV737	CXCR3	1C6/CXCR3								1.3	20.8			
8	V1				BUV805	CD4	SK3						1.5	24				0.5	8.0
9	V3				BV421	CD127	A019D5						1.5	24					
	V3				Pacific Blue	CD14	M5E2								2.0	32.0			
10	V5				Pacific Blue	CD19	SJ25C1								2.0	32.0			
11	V7				BV480	CD161	HP-3G10						2	32					
					BV510	CD45RA	HI100								0.7	11.2			
12	V10																		
13	V11				BV605	CD56	5.1H11						1.0	16				0.1	1.6
14	V13				BV650	CCR7	G043H7						1	16				0.7	11.2
15	V14				BV711	CD7	M-T701						1	16					
16	V15				BV750	IFNγ	B27											1.5	24.0
17	B2				BV786	CCR6	11A9						1.5	24					
18	B3				FITC/AF488	Va24/hCD1d	6B11												
19					Spark blue 550	CD3	SK7					<:10/1.5>			1.2	19.2		0.1	1.6
20	B4				PerCP-Cy5.5	CD26	BA5b											2.5	40.0
21	B6				PE	NKG2D	1D11						1.5	24				0.5	8.0
22	B8				PE-Dazzle594	TNFa	MAB11											1.5	24.0
23	B13				PE-Cy5	CD25	M-A251						1.2	19.2				0.5	8.0
24	R1				PE-vio770	PD1	PD1.3.1.3								1.5	24.0		0.5	8.0
25	R2				APC	CD16	3G8								0.7	11.2			
26	R4				AlexaFluor647	Va7.2/hMR1	3C10					<2:10/1.2>							
27	R6				APC-R700	CD107a	H4A3		6.0	96									
28	R7				Zombie NIR	L/D	N/A				<1:2500>								
29	R8				APC/Fire 750	CD27	O323												
					APC/Fire 810	CD38	HIT2						1.6	32					
														25.6					
And UNSTAINED CONTROLS !!!						Antibody Total			6.0	96	Antibody Total		17.8	285	12.5	200.0		9.5	152
						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	[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						B2	FITC/AF488	[50] Vα24/hCD1d							
582	UV9	BUV563	[55] CD69	V7	BV510	[50] CD45RA	B3	SparkBlue 550	[50] CD3							
598				V8	BV570		B4			YG1	PE	[50] NKG2D				
613	UV10	BUV615	[45] CCR4	V9			B5									
664	UV11	BUV661	[50] Vδ2	V10	BV605	[50] CD56	B6			YG3	PE-Dazzle594	[60] TNFα				
679				V11	BV650	[?] CCR7	B7						R1	APC	[50]	CD16
697	UV12						B8			YG5	PE-Cy5	[40] CD25	R2	AF647	[?]	Vα7.2/hMR1
717	UV13			V12			B9	PerCP-Cy5.5	[45] CD26				R3			
738				V13	BV711	[55] CD7	B10						R4	APC-R700	[50]	CD107a
750	UV14	BUV737	[40] CXCR3				B11						R5			
760				V14	BV750	[50] IFNγ										
783	UV15						B12						R6	Zombie NIR	[50]	Viability
812	UV16	BUV805	[50] CD4	V15	BV786	[50] CCR6	B13			YG9	Pe-Vio770	[50] PD1	R7	APC-Fire 750	[45]	CD27
				V16			B14						R8	APC-Fire 810	[50]	CD38

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