

June 1st, 2023

2023_ILT_05

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
6001 193 1600C 6001 Inf055 ⁰ a-6	HU ♂ RVQ9	Box 3BAS (5)	~9M <6.97			1.0	8.91	13.33	8.91
a-5	RVQ8	(10)	<11.37			1.5	5.71	8.85	8.56
4001 810C Inf278 ⁷ a-3	HEU-10 SEFF	Box 8AH1 (4)	<21.77			0.5	4.52	11.1	
4001 770C a-2	SLFF	(6)	<21.97			0.5	4.43	10.5	
1740C 6001 Inf1595 ⁵ a-7	HEU-hi SBFL	Box 6A A6 (3)	~3M <11.57			1.0	8.64	12.3	
1070C 5001 a-6	SBFL	(8)	~3M <13.87			0.5	6.77	10.7	
1140C 5001 a-8	SBFM	(9)	~3M <12.67			0.5	7.46	11.6	
1200C 6001 Info39 ² a-9	HEU-hi RUTA	Box 2BA4 (4)	~8M <28.77		liquid nitrogen inside massive clump	1.0	2.75	3.05	
830C 4001 ND050	Adult	(2)	ISE6 <12.47	1/13/23		1.0	8.49	11.6	
1130C 4001 ND006	Adult	(1)	ISE6 <6.97	5/12/23		1.0	15.10	18.0	

11:26pm Thaw start
-11:36am 1st cord set
11:53am 2nd cord set
Stain count @ 12:46pm
Count @ 1:00pm
3rd cord set spin @ 2:05pm 2:18pm DNAse
2:36pm aliquots
3:12pm mixes prepared

3:27pm Incubation start → 9:27pm

Reagents prepped @ 8:27pm
9:16 Abs prepped
9:33 sample spin
1/0 @ 9:47 → 10:02pm spin @ 10:00
sc spin 9:53
10:11 sc L/O → 26
10:17 hot scs → 47 spin @ 10:00pm
10:26 samples hot → 56 spin @ 11:02pm
10:36 cold scs → 11:06
S1045 Tet Mixes made
Tets @ 11:18pm
Abs 11:20 → 11:59pm
sc Fix Perm @ 11:25pm → 35 → 45pm

Extra Vials

Inf 278 7B CS-c7
Inf 039 2A c4
→ RUT6 a5

000 | 0 00
00 | 0 00
00 | 0 00
00 | 0 00
00 | 0 00

000 0

1st Perm wash scs @ 11:49pm

combined spin @ 12:07am

12:24pm cold samples → 12:54
12:30am Intra scs → 1:10

Rbelysc @ 12:57am spin @ 1:04am

Fix Perm @ 1:18 → 28 → 38am

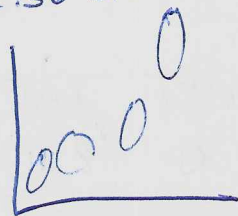
Sc's done @ 1:22pm

Perm 2nd spin @ 1:56am

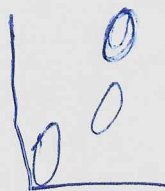
2:09 Intracellular → 2:49pm

Final spin @ 2:50am Done @ 3:01am

12:56 start



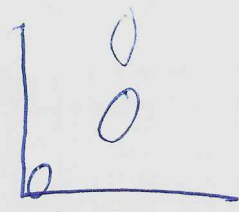
Inf055



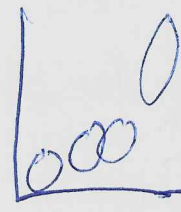
Inf278

more monocyte heavy

27 pl ↑ more PMN-AE staining terminal.

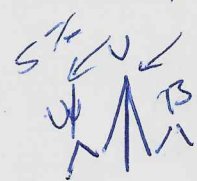


Inf159



Inf039

(2nd specimen at 7)



1:25 Reg₁ samples

57 pl
48 pl
49 pl

Inf039 was within ballpark range # lymphocytes.

Inf055 clump 211 final sample.

Sol's C 2 (14) 3800/50 → 280,000 counts
→ 26 W's
→ 41 Violets
→ Blue/46 (54)
→ Reds 07

22

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100

#	Filter	Single color (u)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Val lot #	During stim!!!	16	U/D 15 min (RT)	Tetramer 40 min @ RT	HoStain 30min @37C	16	ColoStain 30min @4C	16	RBC Lys then FluPerm	Spiked 40 min @RT	16
1	UV/2				BUV335	CD62L	SK411								1.2	19.2			
2	UV/2				AE	AP-UV6													
3	UV/9				BUV486	CD8	RPA-18								0.7	11.2			
4	UV/10				BUV563	CD69	IR540								0.5	8.0		1	16.0
5	UV/11				BUV615	CD44	1G1						2.0	32	0.7	11.2		0.1	1.6
6	UV/14				BUV661	VS2	B6												
7	UV/16				BUV737	CD43	1G6/CD3								1.3	20.8			
8	UV/1				BUV805	CD4	SK3						1.5	24	2.0	32.0		0.5	8.0
9	UV/3				BUV921	CD127	AD1905						1.5	24	2.0	32.0			
10	UV/5				Pacific Blue	CD19	M552								2.0	32.0			
11	UV/7				BUV480	CD161	HP-3610						2	32	2.0	32.0			
12	UV/10				BUV510	CD45RA	HI100								0.7	11.2			
13	UV/1				BUV605	CD56	5.3H11											0.1	1.6
14	UV/13				BUV650	CCR7	GD3H7						1.0	16				0.1	1.6
15	UV/14				BUV711	CD7	M-1701						1	16				0.7	11.2
16	UV/15				BUV750	IFN γ	B27												
17	UV/16				BUV786	CD66	11A9											1.5	24.0
18	UV/1				FTIC/AF488	V α 24/CD1d	6B11											1.5	24.0
19	UV/1				Spark blue 550	CD3	SK7												
20	UV/1				PerCP-Cy5.5	CD38	BA56												
21	UV/1				PE	NKCD20	1D11								1.2	19.2		0.1	1.6
22	UV/1				PE-Dazzle594	TNFR	MAA11						1.5	24				2.5	40.0
23	UV/1				PE-Cy5	CD25	M-A251											0.5	8.0
24	UV/1				PE-A6470	PD1	PD13.13						1.2	19.2				1.5	24.0
25	UV/1				APC	CD16	368								1.5	24.0		0.5	8.0
26	UV/1				Allophycocyanin	V α 7.2/PMR1	3C10								0.7	11.2			
27	UV/1				APC-R700	CD107a	H4A3												
28	UV/7				Zombie NIR	L/D	N/A												
29	UV/8				APC/Fire 750	CD27	C233												
					APC/Fire 810	CD38	HT2												
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		
Pipette draw volume /sample									19.5		Pipette draw volume /sample	65		59.5		19.5			

V α M μ	V α 24/13	1.5	10
ITC	V α 7	1.2	15
AT647	PBS	17.8	12
Pipette draw volume/sample		19.5	178

Tetramer M μ	NC14/PBS-57	0.5	5
AT648	IMM1 5-OP-MU	2	3
AT647			12

Zombie M μ	0.8	18	15	6	Zombie
Number Samples	16	12	10	30	6
Number Unstained	16	12	10	30	6
Number Surface SCs [34]	16	12	10	30	6
Number Intracellular SCs	16	12	10	30	6
Total	16	12	10	30	6

FBS	140	Perm	16.8
PBS	140	Water	151
PFA	0.187	RBC	0.78
PBS	1.883	Water	7.02

Tetramer Control M μ	NC14 Unstained	0.5	1
AT648	IMM1 6-PP	2	0.6
AT647			2.4

Tet PMS	1.9	2.18	3.27
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PBS	1.883	Water	7.02

Simplified Protocol

Thaw cells, DNase, count.
 Gated, count, aliquot cells 2.5 OE-6 Cells R10 / 5ml polypropylene tube
 Bring volume up to "x" ml, R10, add "y" μ l PNAChit and "z" μ l CD137A
 Cap and incubate at 37°C for 6 hours

Wash with 2 ml PBS, spin down 1300rpm 8min
 800 μ l of Live/Dead mix (1:2500) eRT for 15min
 Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min

Add HoStain mix, incubate @37°C for 30 min
 Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min

Add Tetramers, incubate eRT for 10 min
 Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min

Add ColloStain mix, incubate @4°C for 30min
 Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

300 μ l BD FACSPerm, incubate @4°C for 20min

(vortex every 10 minutes)

First PermWash: 1 ml PermWash 1600 rpm 6 min
 Second PermWash: 1 ml PermWash 1600 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min
 First PermWash: 2 ml PermWash 1600 rpm 6 min

Resuspend in 70 μ l 0.4% PFA-PBS

Cap tubes, wrap rack in foil, store at 4°C

[illegible][illegible]

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