

May 26th, 2023

2023_ILT_03

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
Inf018-9 a-4	HU ♂ RLT9	Box 1A (4)	<10.57			1	6.17	9.07	
a-5	RLTA	(10)	<11.67			1	6.31	9.32	
Inf100-1 a-5	HEU-66 RY9L	Box 4A (2)	<20.7>		death spiral	1	3.65	7.99	
a-6	RY9M	(9)			death spiral lysing monocytes	1	4.98	9.67	
Inf134-8 a-6	HEU-10 S711	Box 5A (5)	<11.7>			1	5.84	9.58	
a-5	S710	(7)	<12.7>			1	5.53	8.83	
Inf314-8 a-6	HEU-10 SNHF	Box 8B (6)	<7.6>			1	8.44	12.70	
a-7	SNH6	(8)	<6.1>			1	10.00	14.10	
ND050	Adult	(1)	20E6	1/13/23		1	8.38	12.00	
ND006	Adult	(2)	15E6	1/20/23		1	10.80	13.90	

11:22 adults thaw start
11:34 1st set cord thaw start
Spin @ 11:44 / prep DNAse @ 12:08
11:48 2nd set cord thaw start
Spin @ 12:03 / prep DNAse @ 12:20
12:40 cell stand
1:32 pm aliquot start
2:20 ND006 pmz / p-cps made

4

33	x6
198	

183	x3
549	

1.83

12	x2
24	

3	x2
6	

3M (or close) 72.8

12.5	2.5	0	0	0
11.9	1.4	0	0	0
12.4	8.4	0	0	0
0	0	0	0	0

2:35pm Incubation start

Abs @ 7:43pm
8:03 pm reagents prepared

8:46pm spin

4:00:04pm
Sc spin @ 9:11 pm
9:28 spin
9:33 sc L/O → :48

9:41 hot scs → 10:11 pm

9:50 hot samples → 10:20 pm

22:02 cold scs

spin @ 10:27pm

10:45pm Tets → 11:25

Abs → 10:58 → 11:28 pm

Sc's resuspended 20ul
samples @ 30ul

Fix Perm scs @ 23:05 → 15 → 25

Combined spin @ 11:38 pm

11:55pm samples cold → 00:25

RBC lyse @ 12:27 am
wash @ 12:36

Sc intra @ 12:31 → 1:11 am
Sample Fix Perm @ 12:50 → 00 → 10
1st wash @ 1:17 am

Intracellular @ 1:45 am → 2:25 am

Final spin @ 2:33 am

Pone @ 2:45 am

Mark off the freezer log

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Aurora 5 on @ 4:30 pm.
running H₂O @ 4:49 pm.
QC @ 5:02 pm

0 N 2023

5 sec wait alert, aiming 30 pl (added 20 pl sc's) 150K cells
about a drop left.

<coffee>

6:15 pm restart

6:24 NDOSO → 12,400 events, about 614. 30 pl → 55 pl ← collection.
14,200 events about 800
9600 events about 384
1,350,000 cells
(probably 2 drops left...)

Inf 018

18000

15,200

about 1000

test acc
test accident, added a drop PBS, ran to end,
rescued: 1.7M events cells.

PMA noticeable effect.

Inf 00 mostly dead

24,600

3300

27,000

4800

Inf 134

19,000 / 1900

Inf 314

20,000 / 2000

Done @ 7:39 pm

CO38

ctrl vs

Activated?

CO25 sc?

KZ it's not here.

100



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
INF018	12.48	2	6.24	0.48	0.44	0.40	0.36	0.32	0.28	0.24	0.20	0.16	0.12	0.08
INF100	8.63	2	4.32	0.70	0.64	0.58	0.52	0.46	0.41	0.35	0.29	0.23	0.17	0.12
INF134	11.37	2	5.69	0.53	0.48	0.44	0.40	0.35	0.31	0.26	0.22	0.18	0.13	0.09
INF314	18.44	2	9.22	0.33	0.30	0.27	0.24	0.22	0.19	0.16	0.14	0.11	0.08	0.05
ND050	8.38	1	8.38	0.36	0.33	0.30	0.27	0.24	0.21	0.18	0.15	0.12	0.09	0.06
ND006	10.8	1	10.80	0.28	0.25	0.23	0.21	0.19	0.16	0.14	0.12	0.09	0.07	0.05

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
	INF018	0.52	0.475	0.432	0.39	0.345	0.303	0.26	0.216	0.173	0.13
INF100	0.30	0.279	0.254	0.23	0.203	0.177	0.15	0.126	0.101	0.08	0.051
INF134	0.47	0.432	0.393	0.35	0.314	0.275	0.24	0.196	0.157	0.12	0.079
INF314	0.67	0.618	0.562	0.51	0.449	0.393	0.34	0.280	0.225	0.17	0.113
ND050	0.64	0.588	0.535	0.48	0.427	0.374	0.32	0.267	0.214	0.16	0.107
ND006	0.72	0.661	0.602	0.54	0.481	0.421	0.36	0.300	0.240	0.18	0.121

Sample	PMA										
	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF018	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF100	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF134	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF314	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							Volume	Lym	Total	Average
	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M				
INF018	6	5.5	5	4.5	4	3.5	3				
INF100	6	5.5	5	4.5	4	3.5	3	1	8.44	8.44	
INF134	6	5.5	5	4.5	4	3.5	3				
INF314	6	5.5	5	4.5	4	3.5	3	1	10	10	9.22
ND050	6	5.5	5	4.5	4	3.5	3				18.44
ND006	6	5.5	5	4.5	4	3.5	3				

Volume	Lym	Total	Average
1	8.44	8.44	
1	10	10	9.22
		18.44	

Number Samples	Total	FFS	RBC type	Pix	Pem	PFA
Number Unfinished	16	128	4.8	4.5	64	0.72
Number Surface SCS (34)	12	96	3.2	3.6	24	0.3
Number Intracellular SCS	28	36	5.6	5.6	36	0.7
	6	12	0	1.2	24	0.3
Total		292		8.4	168	1.87

FFS	146	Pem	16.8
PBS	146	Water	15.1
PFA	0.187	RBC	0.84
PBS	1.683	Water	7.56

Perm	16.8
Water	151

Resuspend in 70 μ l 0.4% PFA-PBS

~~CD 50~~ 1.5 SL
CD 27 card
(not CD 38)

Spectrum	UV		Violet		Blue		Yellow-Green		Red	
373	UV1									
388	UV2	BUV395	CD62L							
428	UV3			BV421	CD127					
443	UV4			PacBlue	CD14/19					
458	UV5									
473	UV6	AF								
508										
514	UV7	BUV496	CD8	BV480	CD161	B1				
525										
542	UV8					B2	FTC/AF488			
582	UV9	BUV563	CD69	BV510	CD45RA	B3	SparkBlue 550			
598				BV570		B4		Vα24/hCD1d		
613	UV10	BUV615	CCR4	BV605	CD56	B5		CD3		
664	UV11	BUV661	V62	BV650	CCR7	B6	PE-Dazzle594	TNFα	APC	CD16
679						B7			AF647	Vα7.2/hMR1
697	UV12					B8				
717	UV13			BV711	CD7	B9	PE-Cy5	CD25		
738						B10			APC-R700	CD107a
750	UV14	BUV737	CXCR3	BV750	IFNγ	B11				
760										
783	UV15					B12			Zombie NIR	Viability
812	UV16	BUV805	CD4	BV786	CCR6	B13	Pe-Vio770	PD1	APC-Fire 750	CD27
						B14			APC-Fire 810	CD38

[illegible]



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