

March 21st, 2023

2023_ILT_05

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
Inf0323 a4	HU ♀	(3)	16.45	08/03/18		1.0	5.79	7.52			
a-3	RZFS	(2)	18.37			1.0	5.06	6.84			
Inf100-1 a-3	HEU-1p RZFS	(1)	17.74			1.0	4.62	8.74			
a-2	R49H	(6)			<Dead>	0.5	0.19	6.64 6.64			
Inf314-8 a-5	HEU-1p SWHE	(5)	12			1.5	6.22	9.00			
a-4	SWHD	(7)				1.5	6.90	9.64			
ND050	Adult Norm	(2)	KEG	1/13/23		1.5	5.37	7.37			
ND006	Adult Sc's	(1)	ISEG	1/20/23		1.5	7.24	9.13			
Inf100-1 a-4	R49H Thaw @ 10:30 am	(1)				1.0	6.79	11.5			

und @ 10:30 am
Final Thaw: 11:03
done

11:30 3rd
SWASE

stan @ 11:51 am

2:07 pm

Incubation @ 2:19 pm → 8:19 pm
Brh!

Reagents prepped @ 8:16

Samples spin incl PBS @ 8:16 pm

L/p @ 8:43 pm → 58 pm

Massive spin @ 9:04 pm → 9:12 pm

Hot samples @ 9:30 pm → 10:00 pm Wash @ 10:08

3pc sc's @ 9:40 pm → 10:10 pm Spin @ 10:16 pm

4c @ 9:53 pm → 10:23 pm Spin @ 10:28 pm

Tets @ 10:24 →

Va 7.2 @ 10:36 pm → 11:05 pm

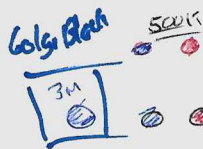
Cells @ 10:43 pm → 53 → 11:03 pm

Combined spin @ 11:16 pm → 22

4c @ 23:30 pm → 00:00

Sc's 2nd Perm @ 11:38 pm

Intra sc's @ 00:03 → 00:43



Inf032: 10.85 % 2

Inf100: 11.61 % 2.5

Inf314: ~~19.68~~ % 3.0

ND050: 8.05 % 1.5

ND006: 10.86 % 1.5

9 + 1.5 = 10.5

9 + 1.5 = 10.5

Samples RBC lyse @ 12:06 am

→ Final FBS wash @ 00:13

Fix Perm @ 12:24 → 34 → 44

1:16 pm Intra → 1:56 am

Final spin @ 2:01 am

Done @ 2:13 am

Jan 1-001 242

1000 (100)



March 21st, 2023

2023_ILT_05

Aurora clean: 2:30 pm

Aurora QC @ 2:48 pm


→ Failed FSC QC @ 9:00 AM....

Passed ✓

Start @ 2:53 pm

38.5C's

1 hr (+3 specimens left)
(+4 min)

There's a bit of a lip on UV1/UV2 today 
★ CD69 debris/clump ★

CD4 (no intra spillover)

CD161 cord is cute ✓

 separation
noise

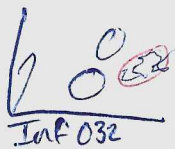
~1700 events

FCS express → gates XML


Samples @ 4:02 pm


60% ~ 2M

ctrl


Inf 032

Done @ 4:58 pm


Lysed specimen?
3.2M eqri
2:22
Inf 100


Inf 3/4

Page 1 of 1

1. Introduction

2. Methodology

3. Results and Discussion

4. Conclusion

5. References

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
INF032	10.85	2	5.43	0.55	0.51	0.46	0.41	0.37	0.32	0.28	0.23	0.18	0.14	0.09
INF100	11.61	2.5	4.64	0.65	0.59	0.54	0.48	0.43	0.38	0.32	0.27	0.22	0.16	0.11
INF314	19.68	3	6.56	0.46	0.42	0.38	0.34	0.30	0.27	0.23	0.19	0.15	0.11	0.08
ND050	8.05	1.5	5.37	0.56	0.51	0.47	0.42	0.37	0.33	0.28	0.23	0.19	0.14	0.09
ND006	10.86	1.5	7.24	0.41	0.38	0.35	0.31	0.28	0.24	0.21	0.17	0.14	0.10	0.07

R10	Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
	INF032	0.45	0.409	0.372	0.34	0.297	0.260	0.22	0.186	0.149	0.11	0.075
	INF100	0.35	0.324	0.295	0.27	0.235	0.206	0.18	0.147	0.118	0.09	0.059
	INF314	0.54	0.497	0.452	0.41	0.361	0.316	0.27	0.225	0.181	0.14	0.091
	ND050	0.44	0.404	0.367	0.33	0.293	0.257	0.22	0.183	0.147	0.11	0.074
	ND006	0.59	0.536	0.488	0.44	0.390	0.341	0.29	0.243	0.195	0.15	0.098

PMA	Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
	INF032	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
	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	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M
	INF032	6	5.5	5	4.5	4	3.5	3
	INF100	6	5.5	5	4.5	4	3.5	3
	INF314	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

2

4

1.13

1.13

0.06

3.3

3 1

18

5

8 H

32

$\overbrace{\text{Vla}}^{\text{2nd} \rightarrow \text{extra}}$
 $\text{1st} \rightarrow \text{inner}$
 CXC 73 Vla end

64 57.5 54

2nd → extra
1st → inner

CCIR3 the end

Bring volume up to 1 ml R10, add 2 ul PMA/Ctrl and CD107a

Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min

Wash 2 ml 5% PBS-FBS 1400 rpm 5 min

Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min

Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

(vortex every 10 minutes)

Second Perm Wash: 1 ml PermWash 1500 rpm 6 min

First PermWash: 2 ml PermWash 1500 rpm 6 min

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

1.5/1.2 antibody

3/21/2023

[illegible]

March 21st, 2023

2023_ILT_05

Unmixing setup

Only ND006 & Inf314 controls / - CD107a PMA ND050C

↔ requires swapping manually *

group → ND050 ctrl/PMA unstained per normal

⚡ Blip CD69 experiment (clot/clump) → (new fsc/ssc pop.)

⚡ difficult to gate on brightest CD56

need to change CD25 to PMA ✓ (already set)

⚡ amine slurry vs rare events

March 21st, 2023

2023_ILT_05

March 21st, 2023

2023_ILT_05

