

July 18th, 2023

2023_ILT_15

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
INF179-7 a-3	HU ♂ SQLA	(5)	<9.457 ~15M			1	9.16	11.5	
Inf149-4 a-3	HU ♀ S7W2	(6)	~9M <11.87 ~6.827			1	6.31	9.41	
a-4 S7W3			<14.187			1	5.45	9.10	
Inf071-8 a-1	HEU-hi ♂ RWM6	(4)	<11.067			0.5	7.94	12.7	
a-2 RWM7		(14)	<9.397			0.5	9.46	15.4	
Inf187-6 a-3	HEU-hi ♂ SALU	(2)	~15M <8.017			1	9.11	15.8	
Inf251-8 a-1	HEU-hi ♀ SFPO	(1)	<12.47		lysed?	1	7.63	10.7	
a-2 SFPI		(15)	<13.987			1	6.84	9.08	
Inf313-7 a-4	HEU-hi ♂ SNFF	(3)	<18.017		LN2 inside?	1	3.63	7.72	
a-3 SNFE		(11)	<17.317		LN2 inside!	1	3.80	8.09	
Inf627-5 a-1	HEU-hi ♀ TJR	(13)			LN2 snapped! not even enough LN2 snapped x 2. Percent w/ 2 vials				
Inf568-9 a-1	HEU-hi ♀ TFDD	(10)	<14.047			1	6.00	10.1	
ND050	Adult ♂	(7)	15E6 11.02	1/13/23		1	8.61	12.0 4.85	
ND006	Adult ♀	(8)	15E6 6.82	5/12/23		1	14.0	16.9	

start @ 9:57 am spin @ 10:12 10:36 dmase resus 10:55
10:56 am spin @ 11:15 11:39 am DNase
12:04 start for count

1:02 fishing extra 313 vial
2:02 aliquot done
2:15 spin
2:41 merge dls

3:07 pm Incubation start

→ 9:07 pm off

Abs done @ 8:42

reagents mostly done @ 9:05 pm

Cells out @ 9:13 pm

< 9:22 pm Cells spin >

sc's spin @ 9:39 pm

1/0 samples @ 9:43 → 58 pm
C:49 for sc's

Hot sc's @ 9:57 pm → 27 pm spin @ 10:05 pm

Cold sc's @ 10:14 pm → 44 pm wash @ 44

Hot samples @ 10:34 pm → 11:00

Tib made before 10:49 pm spin @ 11:09

Sc Fix Pen @ 11:02 → 12 → 22 pm

1440c Inf568-9 HEU-hi ♀ (12) 1ml
spin TFDD a2 <13.217 6.39 10.8

fets @ 11:32 pm → 12:12 am
 Abs @ 11:43 pm → 12:13 am → Wash @ 00:20 am
 2nd se pm was 2 @ 11:39 am
 SC intrae @ 23:53 → 00:33 am → SPN @ 00:36 am
 sc's in 15pl @ 00:03 am
 Cold samples @ 00:40 am → 1:10 am

Sc's done @ 00:45 am

RBC lye @ 1:14 am SPN @ 1:22 am

Fix Perm samples @ 1:37 am → H7 → S7

First wash @ 2:04 am
 2nd @ 2:18 am

2:36 am Intracellular's → 3:16 am

2:42 am unstained's w/ 15pl @ 2:43 am

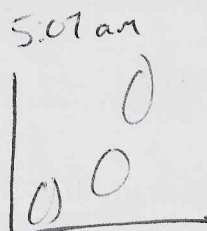
Done @ 3:40 AM

Aurora QC @ 4:02 AM II event rate > 100, $\mu\text{L}/\text{min} \rightarrow 19.69$

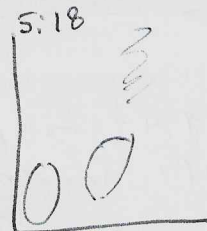
SC @ 4:09 am Cord se monocyte dng relatively healthy SSC-1.

[AF647 CD3 + Va7.2. And 2 unstained partial got ↑ dose ctrl gds. block]
 I before I forget to write down experiment notes.

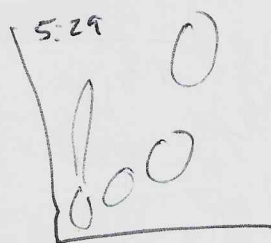
Sc's done @ 5:04 am



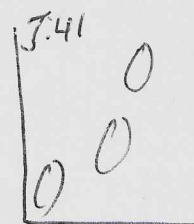
ND056
 14.8K/1K
 60pl 1.9M



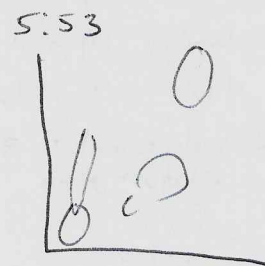
Inf179
 18.5K/1.9K
 70pl 2.6M



Inf149
 18K/1.5K
 70pl 2.5M



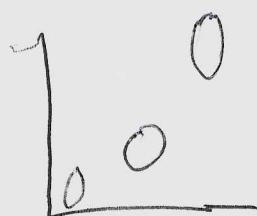
Inf071
 15K/1K
 70pl 2M



Inf187
 18K/1.5K
 65pl 2.5M

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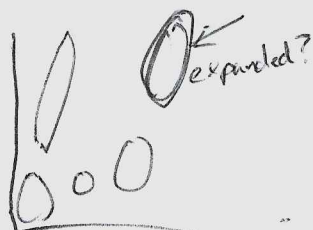
Inf 251

16K / 1.2n

57K / 1.9M

(Amount way up vs prev)

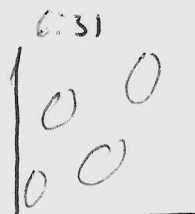
Done 6:41 am



Inf 313 hechi \nearrow

23K / 2.8M

97K / 4.3M?



Inf 568

21K / 2n

69K / 2.35M

Unmixing Card's CP8 \approx AF? signature
similar-ish on ADI, something specimen specific?

Tetramess worked ✓

STATE OF NEW YORK
IN SENATE
January 10, 1901
REPORT
OF THE
COMMISSIONERS OF THE
LAND OFFICE
IN RESPONSE TO
A RESOLUTION PASSED
BY THE SENATE
MAY 1, 1899

ALBANY: THE UNIVERSITY OF THE STATE OF NEW YORK
PRINTING OFFICE, 1899.
✓

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
INF179	9.16	1	9.16	0.33	0.30	0.27	0.25	0.22	0.19	0.16	0.14	0.11	0.08	0.05
INF149	11.76	2	5.88	0.51	0.47	0.43	0.38	0.34	0.30	0.26	0.21	0.17	0.13	0.09
INF071	8.7	1	8.70	0.34	0.32	0.29	0.26	0.23	0.20	0.17	0.14	0.11	0.09	0.06
INF187	9.11	1	9.11	0.33	0.30	0.27	0.25	0.22	0.19	0.16	0.14	0.11	0.08	0.05
INF251	14.47	2	7.24	0.41	0.38	0.35	0.31	0.28	0.24	0.21	0.17	0.14	0.10	0.07
INF313	11.14	3	3.71	0.81	0.74	0.67	0.61	0.54	0.47	0.40	0.34	0.27	0.20	0.13
INF568	12.39	2	6.20	0.48	0.44	0.40	0.36	0.32	0.28	0.24	0.20	0.12	0.12	0.08
ND050	8.61	1	8.61	0.35	0.32	0.29	0.26	0.23	0.20	0.17	0.15	0.12	0.09	0.06

Full PMK
 50
 40
 1.83
 7.32 p1
 3.715
 7.43
 5.573
 11.145

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF179	0.67	0.64	0.560	0.50	0.448	0.392	0.34	0.280	0.224	0.17	0.142
INF149	0.49	0.448	0.408	0.37	0.326	0.285	0.24	0.203	0.163	0.12	0.082
INF071	0.66	0.600	0.546	0.49	0.436	0.382	0.33	0.272	0.218	0.16	0.140
INF187	0.67	0.614	0.559	0.50	0.446	0.391	0.34	0.279	0.223	0.17	0.142
INF251	0.59	0.536	0.487	0.44	0.390	0.341	0.29	0.243	0.195	0.15	0.098
INF313	0.49	0.430	0.175	0.160	0.14	0.127	0.10	0.079	0.064	0.05	0.032
INF568	0.52	0.400	0.472	0.429	0.39	0.343	0.301	0.26	0.214	0.13	0.086
ND050	0.65	0.597	0.543	0.49	0.434	0.380	0.33	0.271	0.217	0.16	0.109

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF179	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF149	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF071	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF187	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF251	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF313	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF568	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

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M	Volume	Lym	Total	Average
INF179	6	5.5	5	4.5	4	3.5	3					0.5	7.43	3.715	
INF149	6	5.5	5	4.5	4	3.5	3					1	7.43	7.43	5.573
INF071	6	5.5	5	4.5	4	3.5	3								
INF187	6	5.5	5	4.5	4	3.5	3								
INF251	6	5.5	5	4.5	4	3.5	3								
INF313	6	5.5	5	4.5	4	3.5	3								
INF568	6	5.5	5	4.5	4	3.5	3								
ND050	6	5.5	5	4.5	4	3.5	3								

Full PMK
 50
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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" μ L PMACtr and "z" μ L 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

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

300 μ l 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 μ l 0.4% PFA-PBS

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

[illegible]

Also done @ 20" H₂

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