

May 16th, 2023

2023\_ILT\_02

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
1330 40 Inf0325 a-5	HU ♀ RRFL	①	9.24			0.75	9.55	12.4	
1024 60 a-5	RRFL	②	16			0.75	5.58	7.4	11.34
1400 60 Inf1483 a-5	HU ♀ S7U6	③	6.07			1	9.34	12.5	
1750 80 a-5	S7U7	④	7.23			1	7.35	10.7	16.69
1030 60 Inf1618 a-4	HEU6 ♀ SBKD	②	9.7			1	9.50	14.6	-9.5
1300 60 Inf1809 a-5	HEU6 ♂ SQNA	④	10.3			1	8.04	11.2	
1360 80 a-4	SQNA	⑤				1	8.05	10.8	16.09
220 40 ND050		①	20EG	1/13/23		1	8.64	11.7	8.67
830 40 ND006		②	15EG 8.7	1/20/23		1	11.1	13.7	11.1

ND006 thaw @ 9:51

@ 10:06 first 4 spin

Card 2nd  
batch thaw @ 10:07

@ 10:20 second 5 spin

DNase @ 10:27 first

@ 10:41 second

Stain Count @ 11:03 am

11:24 successful count (1 clump before)

11:30 Count complete

12:33 PMA to ND06/50

12:40 mixes prepped

Added 400 µl  
CD07a from main  
bottle

12:58 Incubation start → 6:58 pm

200 µl A b 5 prepped @ 6:48 pm (1/4 RBC 5.5 CO2 dose ... 11)

7:10 pm spin

7:26 pm L/D

7:34 pm se spin

7:53 combine sc's

7:55 Hot sc's → 8:09 spin @ 8:33 pm — stacked @ 8:44 pm

8:07 Samples hot → 8:37

8:18 c/d sc's → 8:48 pm

1 / 2M HEU-h: Inf180 given to D. Rathman

○ ○ ○ ○ ○  
 ● ○ ○ ○ ○ ○  
 ● ● ● ○ ○ ○ ○  
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Tch @ 8:57 → 9:37

Abs @ 9:11 → 9:41

SC's FixPerm @ 9:05 → 15 → 25

1st spin @ 9:30 pm

2nd spin @

Combined spin @ 9:47 pm

sc's stacked @ 9:57

10:04 pm cold samples

→ 10:34 pm

Intra sc's @ 10:15 → 55 pm

sc's 20 µl from 20 µl pipette

RBC Lyse spin @ 10:47 pm

Samples FixPerm @ 11:05 → 25

sc's done @ 11:10

Samples 1st perm wash @ 11:29 am

2nd Perm wash @ 11:41 am

Intra @ 11:55 → 12:35 am

NDO50 test @ 12:02 (issue w/ amount)

unstrained w/ 35 pl

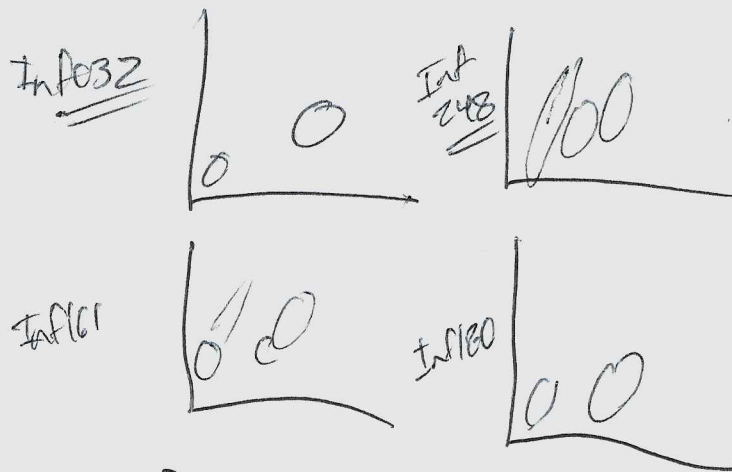
Final spin @ 00:41

Done @ 12:57 am

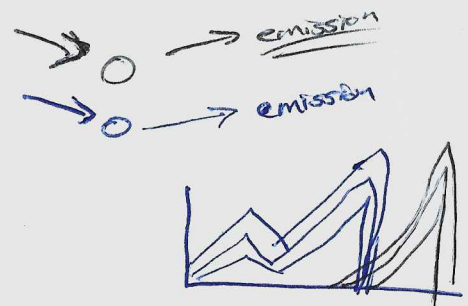
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\* 159,000 2:03 pm start  
Samples c 3:06 pm



Done @ 4:14 pm



no separate input.  
Own unmixing. At  
that point in time, snapshot  
- "Dim channels" ~ similar  
drowned out by other flows  
barring sign over lap.

Unmixing: SeB monocyte ctrl had lysed, low CD62L signal?

\* NO CD16 signal, PMA got swapped into my SC ctrl  
tubes for N0006. \*

not much CD19 either, lot markers impacted.

\* Samples also were PMAd.... \*sigh\* \* at least we have ctrl's  
from prior test run.

Cytokines present, variable effect tetramers, NRTs > resiliency > MPEs





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	11.34	1.5	7.56	0.40	0.36	0.33	0.30	0.26	0.23	0.20	0.17	0.13	0.10	0.07
INF148	16.69	2	8.35	0.36	0.33	0.30	0.27	0.24	0.21	0.18	0.15	0.12	0.09	0.06
INF161	9.5	1	9.50	0.32	0.29	0.26	0.24	0.21	0.18	0.16	0.13	0.11	0.08	0.05
INF180	16.09	2	8.05	0.37	0.34	0.31	0.28	0.25	0.22	0.19	0.16	0.12	0.09	0.06
NDO50	8.69	1	8.69	0.35	0.32	0.29	0.26	0.23	0.20	0.17	0.14	0.12	0.09	0.06
NDO06	11.1	1	11.10	0.27	0.25	0.23	0.20	0.18	0.16	0.14	0.11	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
R10	INF032	0.60	0.552	0.502	0.45	0.401	0.352	0.30	0.251	0.201	0.15	0.101
	INF148	0.64	0.586	0.533	0.48	0.426	0.373	0.32	0.266	0.213	0.16	0.107
	INF161	0.68	0.627	0.570	0.51	0.455	0.399	0.34	0.284	0.228	0.17	0.114
	INF180	0.63	0.574	0.522	0.47	0.417	0.365	0.31	0.261	0.209	0.16	0.105
	ND050	0.65	0.600	0.545	0.49	0.436	0.382	0.33	0.272	0.218	0.16	0.109
	ND006	0.73	0.668	0.608	0.55	0.486	0.425	0.36	0.303	0.243	0.18	0.122

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M	
PMA	INF032	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF148	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF161	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
	INF180	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

[illegible]

12

2 44 44 44

1  
1  
4  
6  
7

3 3 2 2

#	Filter	Single color (ul)	Ref. ctrl	Unmiking ctrl	name	Fluorochrome	Marker	Clone	Vial lot #	During stim	17	U/D 15 min (rt)	Tetramer 40 min @ RT	Hostian 30min @37C	17	ColdStain 30min @4C	17	RBC Lys, FcRperm	Spilled 40 min @RT	17
1	UV2					BUV395	CD26	SK11								1.2	20.4			
2	UV7	Cord				BUV395	CD26	SK11								1.2	20.4			
3	UV9					BUV395	CD8	PR118								0.7	11.9			
4	UV10					BUV395	CD8	PR118								0.5	8.5		1	17.0
5	UV11					BUV395	CD8	PR118								0.5	8.5			
6	UV12					BUV395	CD8	PR118								0.5	8.5			
7	UV13					BUV395	CD8	PR118								0.5	8.5			
8	UV14					BUV395	CD8	PR118								0.5	8.5			
9	UV15					BUV395	CD8	PR118								0.5	8.5			
10	UV16					BUV395	CD8	PR118								0.5	8.5			
11	UV17					BUV395	CD8	PR118								0.5	8.5			
12	UV18					BUV395	CD8	PR118								0.5	8.5			
13	UV19					BUV395	CD8	PR118								0.5	8.5			
14	UV20					BUV395	CD8	PR118								0.5	8.5			
15	UV21					BUV395	CD8	PR118								0.5	8.5			
16	UV22					BUV395	CD8	PR118								0.5	8.5			
17	UV23					BUV395	CD8	PR118								0.5	8.5			
18	UV24					BUV395	CD8	PR118								0.5	8.5			
19	UV25					BUV395	CD8	PR118								0.5	8.5			
20	UV26					BUV395	CD8	PR118								0.5	8.5			
21	UV27					BUV395	CD8	PR118								0.5	8.5			
22	UV28					BUV395	CD8	PR118								0.5	8.5			
23	UV29					BUV395	CD8	PR118								0.5	8.5			
24	UV30					BUV395	CD8	PR118								0.5	8.5			
25	UV31					BUV395	CD8	PR118								0.5	8.5			
26	UV32					BUV395	CD8	PR118								0.5	8.5			
27	UV33					BUV395	CD8	PR118								0.5	8.5			
28	UV34					BUV395	CD8	PR118								0.5	8.5			
29	UV35					BUV395	CD8	PR118								0.5	8.5			
And UNSTAINED CONTROLS III																				
Antibody Total										6.0	102								9.5	161.5
R10 Media										14.5	246.5								11	187
Pipette draw volume / sample										19.5										
Antibody Total										6.0	102								9.5	161.5
R10 Media										14.5	246.5								11	187
Pipette draw volume / sample										19.5										

Tetramer Mix																					
AT488	ICD10 PBS 5/7	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
AT647	NM1 5.0P RU	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	

① ①

...01-

CH 2 300  
CO 2 400  
1001

... 0300 K



[illegible]

Viability	[50]	[45]	[50]
CD27			
CD38			

CD16  
Vα7.2/hMMF

CD107a

AF647

PC-R700

### C-Fire 75



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