

2023_ILT_12

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
Inf7861 a-1	HU ♀ TZQJ	①	<31.3>		lysed? clump few monocytes	1	2.88	3.49	
a-2	TZQK	⑩	<15.5>		lysed? clump few monocytes	1	6.05	7.80	
Inf523-0 a-1	HEU-lo TCU	③	<13.4>			1	6.79	7.11 11.4	
a-2	TCU1	⑨	<12.6>			1	6.85	11.1	
Inf661-3 a-2	HEU-lo TLPX	②	<8.5>			1	9.24	13.6	
a-1	TLPV	⑧	<7.3>			1	11.50	16.9	
Inf318-2 a-1	HEU-hi SNR2	④	<13.4>			1	7.1	11.4	
a-2	SNR3	⑪	<16.9> <16.9>			1	5.79	10.9	
ND050	Adult ♂	⑥	<?>			1	8.14	11.3	
ND006	Adult ♀	⑤	15E6 <7.3>	5/12/23		1	13.1	16.4	
Inf574-6 a-2	HU IFR1	⑦	<20.3>			1	4.71	7.56	

11:13 am show start 11:20 spin DNA₁₂ 011:46

11.29 am All 4 specimens very little blood.

10.31 um adult hawk 10H1 spin

12:07 am 2nd Hour's: Spin @ 12:25 DNAse @ 12:45

1:05 pm start for camp

1:57 pm a/q. of start \rightarrow 14:22 pm

2:46 pm a made

501/ In8574-6 HU (12) 1m1 4.52 7.34
750c TFR Ø a-1 <20.9>

NDWG $0.23 + 0.77 \frac{\text{RPM}}{67} + 2 \text{ per } 5 \text{ min}$

19 tubes

Inoculation C 3:06 pm \rightarrow 9:06 pm

Abs prepped @ 7:30 am

Reagents. prepped @ 7:44pm

Spin samples @ 9:11 pm ✓

1 chl ND006 tube still pink \sim \sim \sim

L/O @ 9:27pm \rightarrow spin @ 9:48pm

SC Spm @ 1:34

→ Low CD 107a to Zambie NIR ...

9:56pm SC for Zombire →

2200pm samples hot \rightarrow 10.35pm spin @ 10.42am

22:12 pm hot sc's \rightarrow 42 pm spin @ 10:45 pm

10:41 pm cold SCS \rightarrow 51 pm

Tets prepped @ 22:28 pm

Abs 11.12

Tets @ 11:00 pm \rightarrow 11:41 pm

11:08 pm SC Fix Perm $\rightarrow 18 \rightarrow 28$

1st magh @ 9:32pm

2nd @ 11:52 pm

→ samples spin @ 11:47pm

Cold samples @ 12:02 am $\rightarrow 32$

Intra SC @ 00:13 \rightarrow 00:53 am

mark sc's started down 00:22

12:45 am last FBS wash

Samples FixPerm @ 1:01 → 10 → 1:21 pm

sets done @ 1:05 am

1st wash @ 1:24 am

2nd @ 1:38 am

Intrac @ 1:53 → 2:33 am

Final spin @ 2:35 am

Done @ 2:50 pm

25 pl samples

15 pl for SC's/unstained

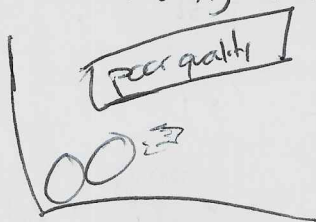
SC acq @ 4:10 pm
done @ 5:01 pm

ND006 Δ color for SC probably cap not Affeny
Δ temp / Δ in CO2 Δ m coloration

Samples @ 5:08 pm
→ mostly dead

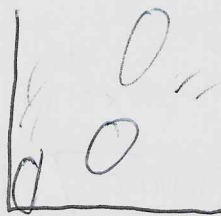
Inf 315 - ctrl unstained
Actually Inf 661 ctrl

overlaid
unmixing
by Δ controls?



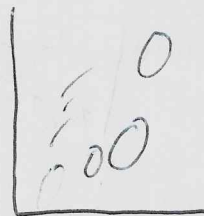
Inf 786

13.4K / 0.8K



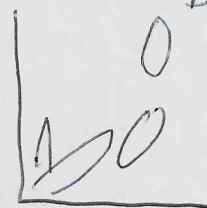
Inf 574

20K / 2K



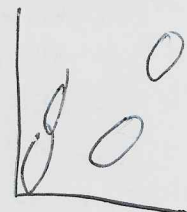
Inf 523

22K / 25K



Inf 661

24K / 3.5K



Inf 318

21.4K / 2.2K

Done @ 6:05 pm

Monocyte FSC/SSC position,
health, activation, organ?

"In (numbers similar looking cells) we trust"

July 5th, 2023

2023_ILT_12

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
INF786	8.93	2	4.47	0.67	0.62	0.56	0.50	0.45	0.39	0.34	0.28	0.22	0.17	0.11
INF574	9.23	2	4.62	0.65	0.60	0.54	0.49	0.43	0.38	0.33	0.27	0.22	0.16	0.11
INF523	13.64	2	6.82	0.44	0.40	0.37	0.33	0.29	0.26	0.22	0.18	0.15	0.11	0.07
INF661	20.7	2	10.35	0.29	0.27	0.24	0.22	0.19	0.17	0.14	0.12	0.10	0.07	0.05
INF318	12.89	2	6.45	0.47	0.43	0.39	0.35	0.31	0.27	0.23	0.19	0.16	0.12	0.08
ND050	8.14	1	8.14	0.37	0.34	0.31	0.28	0.25	0.21	0.18	0.15	0.12	0.09	0.06

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF786	0.33	0.300	0.273	0.25	0.218	0.191	0.16	0.136	0.109	0.08	0.055
INF574	0.35	0.320	0.291	0.26	0.233	0.204	0.17	0.145	0.116	0.09	0.059
INF523	0.56	0.513	0.466	0.42	0.373	0.326	0.28	0.233	0.186	0.14	0.094
INF661	0.716	0.650	0.591	0.53	0.473	0.414	0.36	0.295	0.236	0.18	0.119
INF318	0.5315	0.489	0.445	0.40	0.356	0.311	0.27	0.222	0.178	0.13	0.089
ND050	0.63	0.578	0.526	0.47	0.420	0.368	0.32	0.262	0.210	0.16	0.106

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF786	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF574	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF523	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF661	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF318	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	Volume	Lym	Total	Average
INF786	6	5.5	5	4.5	4	3.5	3	1	9.24	9.24	
INF574	6	5.5	5	4.5	4	3.5	3	1	9.24	9.24	
INF523	6	5.5	5	4.5	4	3.5	3	1	11.5	11.5	
INF661	6	5.5	5	4.5	4	3.5	3	1	11.5	11.5	
INF318	6	5.5	5	4.5	4	3.5	3	1	11.5	11.5	
ND050	6	5.5	5	4.5	4	3.5	3	1	11.5	11.5	
CD107a											10.37
										20.74	

1.83 1.83
 x 6.30 x 3.15
 11.98 5.49
 1.00
 1.83 1.83
 x 6.30 x 3.15
 11.98 5.49
 1.00
 1.83 1.83
 x 6.30 x 3.15
 11.98 5.49
 1.00
 1.83 1.83
 x 6.30 x 3.15
 11.98 5.49
 1.00

SPD1
 *NY620
 *C056
 *V024In18

#	Filter	Single color (u)	Red Cell	Unmixing cell name	Fluorochrome	Marker	Clone	Val loc #	During stim (t)	19	U/D 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @ RT	19	ColdStain 30min @ 4C	19	862 Live FcyRperm	Spiked 40 min @RT	19
1	UV2				BUV395	CD63	SK11								1.2	22.8			
2	UV7				AP	AP-UV6													
3	UV3				BUV468	CD8	RP4-18								0.7	13.3			
4	UV10				BUV463	CD89	FM50								0.5	9.5		1	19.0
5	UV11				BUV615	CD4	1G1												
6	UV1				BUV661	VZ2	B6						2.0	38	0.7	13.3		0.1	1.9
7	UV16				BUV737	CD43	1G6/CD43								1.3	24.7			
8	V1				BUV805	CD4	SP3												
9	V4				BUV421	CD127	AO1305						1.5	28.5				0.5	9.5
10	V3				Pacific Blue	CD14	MS12						1.5	28.5					
11	V5				Pacific Blue	CD19	SJ23C1								2.0	38.0			
12	V7				BUV440	CD161	HP-3G10								2.0	38.0			
13	V10				BUV510	CD45RA	HL100						2	38	0.7	13.3			
14	V11				BUV605	CD56	5.H111												
15	V14				BUV650	CD87	GD34117						1.0	19			0.1	1.9	
16	V15				BUV711	CD7	M-1701						1	19			0.7	13.3	
17	B2				BU786	IFN γ	B27												
18	B3				Fluor/AF488	Vz24/ICD1d	11A9											1.5	28.5
19	B3				Spark blue 550	CD3	SK7												
20	B4				PerCP-Cy5.5	CD26	BASb												
21	B6				PE	TM64	1D11								1.2	22.8		0.1	1.9
22	B8				PE-Dazzle594	TM64	1D11											2.5	47.5
23	B11				Per-Cy5	CD25	M-A251						1.5	28.5			0.5	9.5	
24	B11				PE-46070	PD1	PD13.1.3											1.5	28.5
25	B2				APC	CD16	3G8						1.2	22.8			0.5	9.5	
26	B4				AlloX-fluoroc47	Vz24/ICD1d	3C10								1.5	28.5		0.5	9.5
27	B6				APC-R700	CD107a	1H43								0.7	13.3			
28	B2				Zombie NIR	U/D	N/A												
29	B8				APC/Fire 750	CD27	0323												
					APC/Fire 810	CD38	HL7												
And UNMIXED CONTROLS (H)								Antibody Total	6.0	114	Antibody Total	17.8	338	12.5	237.5	9.5	180.5		
								R10 Media	14.5	275.5	Brilliant Stain	50	950	50.0	950	11	209		
								Pipette draw volume /sample	19.5		Pipette draw volume /sample	65		59.5		19.5			

Tetramer Control Mix				
At-488	ICD1d Unlabeled	0.5		1
At-647	IMM16-IP	2		2.4

Tetramer Mix				
At-488	ICD1d PBS-27	0.5		6
At-647	IMM16-OP-40	2		14.4

Pipette draw volume/sample				
At-488	ICD1d PBS-27	1.5		12
At-647	IMM16-IP	1.2		18
	PBS	17.8		213.6
		19.5		

Zombie mix				
At-488	ICD1d PBS-27	1.5		12
At-647	IMM16-IP	1.2		18
	PBS	17.8		213.6
		19.5		

Zombie mix				
At-488	ICD1d PBS-27	1.5		12
At-647	IMM16-IP	1.2		18
	PBS	17.8		213.6
		19.5		

Number Samples	Total	FBS	RBC lyle	Fix	Perm	PFA
Number Unstained	19	152	5.7	5.7	76	0.57
Number Surface SCs (20)	10	40	3	2	20	0.58
Number Intracellular SCs	29	79	0	5.8	58	0.58
	6	6	0	1.2	24	0.17
Total	6	227	8.7	14.7	178	1.57

204 *204* 160.2 *160*

FBS	Perm	PFA
113.5	178	
113.5	160	

PFA	RBC
0.157	0.87
1.413	7.83

PBS	Water

Simplified Protocol

Thaw cells, DNAse, count

Collect count: aliquot cells 2.0E+6 Cells RT0 / sent polystyrene tube

Bring volume up to 1×10^6 RT0, add 1×10^6 PI/ACtH and 2×10^6 CD107a

Cap and incubate at 37°C for 6 hours

Wash with 2 ml PBS, spin down 1300rpm 8min
800 ul of Liverbed mix (1:2500) @RT for 15min

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

Add HoeStain mix, incubate @37C for 30 min

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

Add Tetramine, incubate @RT for 10 min

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

Add CoulStain mix, incubate @ 4C for 30min

Add 300-500 ul 1% RBC Lysis for 3 minutes

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

300 ul BD FICPerm, incubate @ 4C for 20min

(vortex every 16 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 ul 0.4% PFA-PBS

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

1000s pippped @ 7:30pm

Reagents pippped @ 7:44pm

Ab's pipped @ 7:30 pm
Rosa's pipped @ 7:44 pm

$$\begin{array}{r}
 3 \\
 3 \quad * 3 \\
 \hline
 4 \\
 \hline
 4 \\
 \hline
 3 \\
 \hline
 17 \\
 * 3 \\
 \hline
 54
 \end{array}$$

[illegible]

Spectrum	UV		Violet		Blue		Yellow-Green		Red	
373	UV1									
388	UV2	BUV395	CD62L	BV421	CD127					
428	UV3			PacBlue	CD14/19					
443	UV4									
458	UV5									
473	UV6	AF								
508										
514	UV7	BUV496	CD8	BV480	CD161	B1				
525										
542	UV8					B2	FITC/AF488			
582	UV9	BUV563	CD69	BV510	CD45RA	B3	SparkBlue 550			
598				BV570		B4		Vα24/hCD1d CD3		
613	UV10	BUV615	CCR4	BV605	CD56	B5				
664	UV11	BUV661	Vβ2	BV650	CCR7	B6	PE-Dazzle594			
679						B7				
697	UV12					B8				
717	UV13			BV711	CD7	B9	PE-Cy5			
738						B10				
750	UV14	BUV737	CXCRC3	BV750	IFNγ	B11				
760										
783	UV15									
812	UV16	BUV805	CD4	BV786	CCR6	B12 B13 B14	Pe-Vio770	PD1	Zombie NIR APC-Fire 750 APC-Fire 810	Viability CD27 CD38

July 5th, 2023

2023_ILT_12

July 5th, 2023

2023_ILT_12

July 5th, 2023

2023_ILT_12

