

March 28th, 2025

March Bloodbath

Specimen	Status	Location	Conc	Date	Notes	Volume	CD45+	Total	Resuspension
15/1 Inf687-1	HEU60♀	9B, B5			Dying @ thaw	0.5		0.72	—
15/1 Inf769-2	HEU60♀	11A, F6			OK	0.5		5.0	↑ 1.2
15/1 Inf937-9	HU ♂	14A B5			OK	0.5		2.8	↑ 0.43
15/1 Inf946-9	HU ♀	13B, A1			OK	0.5		6.6	↑ 1.7

1:05 pm Thaw

Inf946-9 PPD ○ PPD
 Inf937-9 SEB ○ Activated SC's
 Inf769-2 ○ 1.7mL Unstim SC's
 Inf687-1 ○ ← Zombie / AF

1:55 pm Spin

2:38 pm Rest

9:25 pm Stim

5:18 AM Golgi @ @

8:00 AM AB prep

Samples out @ 8:24 AM

8:32 spin

L/B @ 8:47 AM → 9:02 spin @ 9:04 AM

Hot SC's @ 8:52 → 9:22 @ 9:27 AM

Cold SC's @ 8:57 → 9:27

1st batch F.S. Hot @ 9:14 AM → 9:44

9:27 AM 2nd batch spin

9:35 AM 2nd batch live/dead

9:54ish Cold for F.S #1 → 10:24 AM @ 10:25 spin

10:02 AM 2nd batch Hot Stain → 10:32 @ 10:34 spin

10:41 → 10:51 → 11:01 → 11:11

10:43 #2 cold stain → spin 11:15 → 2nd fix @ 11:27am

1st perm wash SC's + #1: 11:15

2nd perm wash @ 11:31

Intracellular @ 10:44 → 12:24 pm

2nd 12:16 pm Intracellular 12:16 pm - 12:56pm

0.98 → 0.0811
 % PCV ... allhh

1st Perm
 2nd Perm
 Intracellular

SC's resuspended in Sept @ 11:50 AM

11:27am 11:37am 11:47am 11:57am

March 28th, 2025

18 tubes \times 2 washes @ 500 = 18
5 tubes \times 4 washes @ 500 = 10
2 tubes \times 4 washes @ 1000 = 8

36 mL wash
3.6 mL \rightarrow 32.4 mL Water

23 tubes \times 250 μ L
 \times 2 tubes \times 500 μ L
Fix \rightarrow 5.75
Fix \rightarrow 1.00
7.00 mL

1.75 mL Fix
5.25 mL D-Treat

3rd wash Batch # 1 @ 12:25

4th @ 12:34 PM

Done @ 12:44

2nd Batch at @ 12:57 AM

12:58 3rd wash

March 28th, 2025

#	Filter	Fluorochrome	Marker	Clone	Dose	During stim!!!	2	L/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	2	ColdStain 30min @4C	2	4°C for 15 min	RBC Lysis then FcyPerm	Intranuclear Stain 40 min @RT	2
1	UV1	BUV395	CD62L	(DREG-56)	1							1.2	2.4			0.8	1.6
2	UV2	BUV563	CD69	(FMSO)	2					1.8	3.6	0.5	1			0.1	0.2
3	UV3	BUV615	CD84	(1G1)	2							0.5	1			0.2	0.4
4	UV4	BUV661	CD38	(B6)	2							0.5	1				
5	UV5	BUV737	CD4	(SK3)	1					1.5	3						
6	UV6	BUV786	CD14	(M5E2)	2							1.5	3				
7	UV7	Pacific Blue	CD19	(HB19)	2							1.5	3				
8	UV8	BUV480	CD161	(REA631)	1					1.5	3			Strep <0.4>			
9	UV9	BUV570	CD16		1							0.9	1.8				
10	V10	BUV605	CD45RA	(1252D4)	1							0.9	1.8				
11	V11	BUV650	CD8		1							0.9	1.8				
12	V12	BUV711	IFN γ	(927)	2							1.0	2				
13	V13	BUV750	IFN γ	(11A9)	1							1.2	2.4				
14	V14	Alexa Fluor 488	FoxP3		Beads					1.2	2.4					1.5	3
15	B3	Spark Blue 550	CD3	(SK7)	1							1.2	2.4			0.2	0.4
16	B4	Spark Blue 574	CD45		1							0.6	1.2				
17	B6	RB613	PD1		1												
18	B7																
19	B8																
20	B9	RB705	CD26	(M-A261)	1							1.0	2				
21	B10																
22	B11	RB780	CXCR5		2					1.2	2.4					<0.2>	
23	B12	PE	ICOS	(EH12.2H7)	1											1.5	
24	B13	PE-Dazl 594	TNFR	(MAB11)	2											1	2
25	B14	PE-Cy5	CXCR3		2												
26	B15	PE-Fire 700	CD127		1					1	2						
27	B16	PE-Fire 744	CD25		2							1.5	3				
28	B17	PE-Vio 770	HLA-DR	(L243)	1					1.2	2.4	0.6	1.2				
29	B18	PE-Fire810	CD39	(A1)	1							<1.2>					
30	B19	APC	CD39	(MO1)	1					1.2	2.4						
31	B20	Alexa Fluor 647	IL2		2												
32	B21	APC-R700	CD107a	(H4A3)	1.2	2.4										1.2	2.4
33	B22	Zombie NIR	L/D		2												
34	B23	APC-Fire 750	CD27	(O323)	1												
35	B24	APC-Fire 810	CCR7		1												
36	B25	And UNSTAINED CONTROLS !!!															
37	B26	Antibody Total	1.2														
38	B27	Antibody Total	19.3														
39	B28	Brilliant Stain	25.2														
40	B29	Pipette draw volume	19.5														
41	B30	Antibody Total	12.6														
42	B31	Brilliant Stain	25.2														
43	B32	Pipette draw	34.8														
44	B33	Antibody Total	15.6														
45	B34	Brilliant Stain	31.2														
46	B35	Pipette draw	43.8														
47	B36	Antibody Total	9.5														
48	B37	Brilliant Stain	19														
49	B38	Pipette draw	25.5														

$\alpha\beta$ T cell SFC panel

Wash with 2 ml PBS, spin down 1300rpm 8min
800 ul of LiveDead mix (1:2500) @RT for 15min
Wash 1.5 ml 5% PBS-FBS, spin 1300 rpm, 8min

Add CD161 biotin antibody for 10 minutes at RT

Add HotStain mix, incubate @ 37C for 30min
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min

Add ColdStain mix, incubate @ 4C for 30min
Add 300-500 ul 1x RBC lysis for 3 minutes
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min

Add Streptavidin Mix, incubate @ 4C for 15min
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min

0.8 ml Nuclear FixPerm, incubate @ 4C for 30min
(vortex every 10 minutes)

First PermWash: 1.5 ml NuclearWash 1500 rpm 6 min

Second Perm Wash: 1.5 ml NuclearWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min
First PermWash: 1.5 ml NuclearWash 1500 rpm 6 min
Second Perm Wash: 1.5 ml NuclearWash 1500 rpm 6 min
Resuspend in 80 ul 0.4% PFA+BS
Cap tubes, wrap rack in foil, store at 4C

3/28/2025

3/28/2025									
Spectrum	UV		Violet		Blue		Yellow-Green		Red
373	UV1								
388	UV2								
428	UV3	BUV395							
443	UV4		BY421/						
458	UV5		cFluor V420						
473	UV6		Pacific Blue	CD14/19					
508	UV6	cFluor UV440	SytoxBlue						
514	UV7		BY480	CD161					
525	UV7	BUV495							
542	UV8		SBV515						
582	UV8	BUV563	BY570	CD16					
598	UV9		CDet 385						
613	UV10	BUV615		CD45RA					
664	UV11	BUV661	BY605	CD8					
679	UV11		BY650						
697	UV12								
717	UV13		BY711	Va7.2					
738	UV13								
750	UV14	BUV737	BY750	IFNy					
760	UV15								
783	UV15		BY786						
812	UV16	BUV805		CCR6					

March 28th, 2025

1:05 pm Thaw

1:55 pm Seaw

687 No 0.72 M
63 737 5.0 M
937 2.8 M
6 941 6.6 M
↑ 1.2
↑ 0.43
↑ 1.7

8:38 pm

8:38 pm

↑ 16:38 pm
→ 7:38 AM
9 AM

Thaw

8 hrs
5 min 40

9:25 pm
→ 5:18 am
7:30
8:30
respectively

3:44

Inf 946	7 + 8 hr	1ml	20 μ L
Inf 739	Ctrl	1.7ml	1.7 μ L
Inf 937	SFB	1	10 μ L
Inf 687	20mbz + AF		7 μ L

54 μ L

0.35 W
 0.30 W
 0.28 W
 0.25 W
 0.20 W
 0.15 W
 0.10 W
 0.05 W
 0.00 W

0.35 W
 0.30 W
 0.28 W
 0.25 W
 0.20 W
 0.15 W
 0.10 W
 0.05 W
 0.00 W

0.35 W
 0.30 W
 0.28 W
 0.25 W
 0.20 W
 0.15 W
 0.10 W
 0.05 W
 0.00 W