

November 8th, 2024

CD8 ~~RL~~ BUESO vs SB ^{path}loc 574
 USRA on SY220 or BUSID

301

Specimen	Status	Location	Conc	Date	Notes	Volume	CD45+	Total	Resuspension
NY070	Adult		15E6	4/11/23				15E6	

Friday

Start @ 1:12pm
 Spn @ 1:21pm
 In ab @ 10:40pm → 7:40pm
 SEB m @ 8:00pm
 Stm @ 10:05pm

ctrl SEB ctrl SEB
 O O O O
 2M 2M 2M 2M

↔
 stain each
 then split
 last minute
 @ cold
 step for CD45RA
 2 CD8 (save Abs)

Saturday

Spn @ 11:02pm
 12:57pm Abs prep (HLA-DR, PSD, APC)
 SCs @ 1:01 aliquots off on aliquots
 1:18 spinning

4 spec

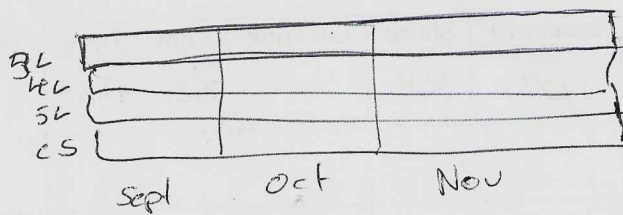
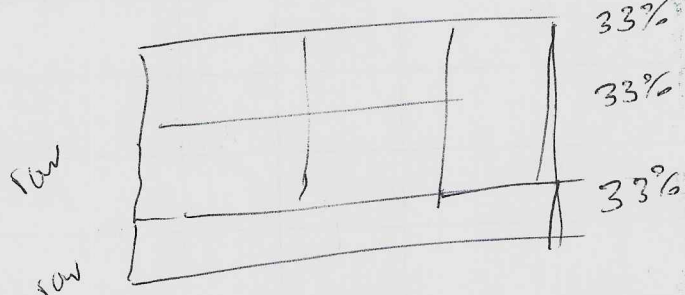
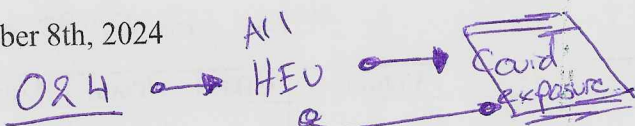
1:33pm Screening Time

2:17pm reagent
 2:24 cold — 2:54pm
 2:32 hot → 3:02 hot wash @ 301
 2:37 samples to → 2:52pm
 3:10pm Hot stain → 3:40pm
 3:22 → 32 → 42 → 54
 3:57 cold → 4:27

Fix
 SCs

First x wash @ 4:03pm
 SCs intra @ 4:33 & spn @ 5:10 2nd @ 5:29pm
 Strep + samples @ 4:38pm → 5:10
 Samples Fix @ 5:03 → 13 → 23 → 33
 1st wash 2nd @ 5:44
 Intra @ 5:53pm → 6:33pm
 Done @ 6:55pm

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Acq @ 7:25pm 40pl + residual for scs
(few if any more fcs)

unmixed @ 9:40pm [16.1] complete

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αβ T cell SFC panel

#	Filter	Fluorochrome	Marker	Clone	Dose	During stim!!!	2	U/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	2	Coldstain 30min @4C	2	4°C for 15 min	RBC Lys, then FixPerm	Intracellular Stain 40 min @RT	2
1	UV2	BUV395	CD62L	(DREG-56)	1							1.2	2.4				
2	UV9	BUV563	CD69	(FN50)	2												
3	UV10	BUV615	CD69	(1G1)	2					1.8	3.6	0.5	1			0.8	1.6
4	UV11	BUV661	CD69	(86)	2							0.5	1			0.1	0.2
5	UV14	BUV737	CD38	(SK3)	1							0.5	1			0.7	1.4
6	UV16	BUV805	CD4	(SK3)	1					1.5	3	1.5	3				
7	V1	BUV421	CD127	(N5E2)	1					1.5	3	1.5	3				
8	V3	Pacific Blue	CD19	(H1B19)	2							1.5	3				
9	V5	BUV480	CD161	(REA631)	1					1.5	3	0.5		Strep <0.4>			
10	V7	BUV510	CD45RA	(H1100)	1												
11	V8	BUV570	CD45RA	(H1100)	1							0.5					
12	V10	BUV605	CD45RA	(H1100)	1					2.5	5	1.0					
13	V11	BUV650	CD8	(V252D4)	2							1.0					
14	V13	BUV711	CD8	(V252D4)	1							1.2	2.4			1.5	3
15	V14	BUV750	CD8	(V252D4)	2							1.2	2.4			0.5	1
16	V15	BUV786	CD8	(V252D4)	1							1.2	2.4				
17	B3	Spark Blue 550	CD3	(SK7)	1					1.2	2.4	1.2	2.4				
18	B4	Spark Blue 574	CD8	(SK7)	1							1.2	2.4				
19	B6	R8613	PD1	(M-A261)	1					1	2						
20	B9	PerCP-Cy5.5	CD26	(M-A261)	1												
21	B14	StarBright 6810	CD26	(M-A261)	1												
22	YG3	PE	ICOS	(EH1.2.2H7.1)	1					1.5	3					2	4
23	YG5	PE-Dazzle 594	TNFA	(MAB11)	2											1	2
24	YG7	StarBright 720	CD45RA		2					1	2	1.0					
25	YG8	PE-Fire 744	CD25		1					1.2	2.4	0.6					
26	YG9	PE-Vio 770	HLA-DR	(1243)	1												
27	YG10	PE-Fire 810	HLA-DR	(1243)	1												
28	R1	APC	CD39	(A1)	1												
29	R2	Alexa Fluor 647	IL2	(MOJ-2)	1					1.2	2.4						
30	R4	APC-R700	CD107a	(H4A3)	2											1.2	2.4
31	R7	APC-Fire 750	CD27	(O323)	1							1.5	3				
32	R8	APC-Fire 810	CD27	(O323)	1												
And UNSTAINED CONTROLS !!!																	
			Antibody Total	1.2						1	2	13.2	26.4			10.8	21.6
			R10 Media	19.3						33.8	67.6	26.4	52.8			21.6	43.2
			Pipette draw volume	19.5						47.7		36.6				29.4	

34
12

32 + 3 = 35
30
5
30 * 1.5 ml = 52 ml FBS wash
30 * 1.6 = 48.0
5 * 3.2 = 16.0
14.0 ml
24.0
78.0 ml

24.0
40.8

78.0 ml

Wash with 2 ml PBS, spin down 1300rpm 8min
800 ul of Live/Dead 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-PBS
Cap tubes, wrap rack in foil, store at 4°C

W
24

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