

December 6th, 2024

Specimen	Status	Location	Conc	Date	Notes	Volume	CD45+	Total	Resuspension
NY02B	Adult		2SE6	8/3/2011	Adult thymus, islet out	2			

10:40 am start

11:06 am DNAse

11:14 am stain count

11:32 am → 5:39 pm

3:10 "F.s"

3:26 "Sc's"

Wash @ 3:36 F.s

4:07 Done

4:30 → 6:59 2 1/2 hrs

3 M ~ 2 Million / 1 ml

7:10 pm

Golgi Plug Added

SEB

15 µL

1 mg/mL

11:32 am spin down

105B DPUCed & stacked 1.2 ml

1:16 pm Abs prepped

1/0 @ 1:17 Spin @ 1:35 pm

second stain @ 1:27 pm spin @ 2:01

Hot stain @ 1:33 pm spin @ 2:07

FS Hot @ 1:52 pm → 2:22 pm

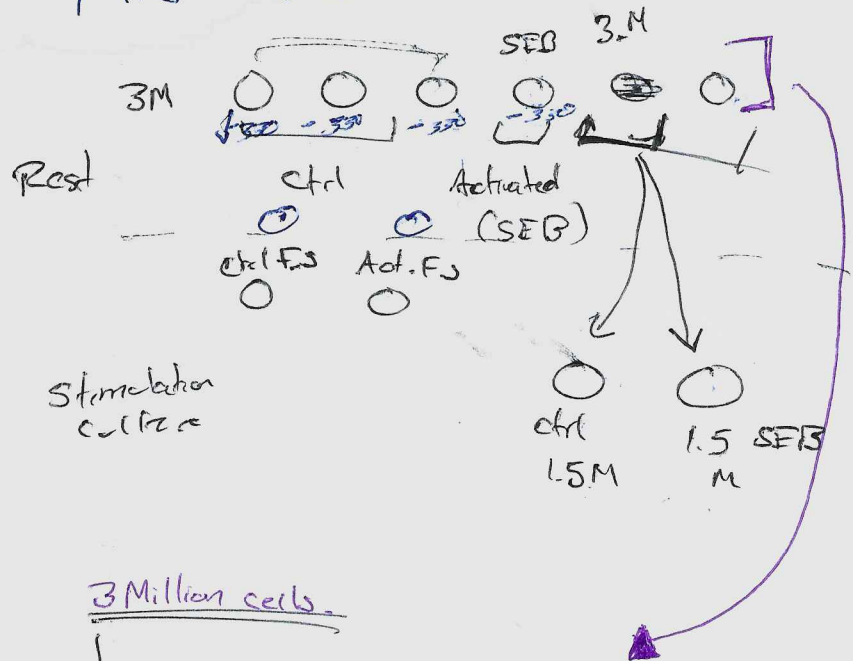
FS cold @ 2:30 → 3:00 pm

Sc's Fix @ 2:35 → 45 → 55 → 3:05 pm

strep @ 3:10 pm

↑ 4.3

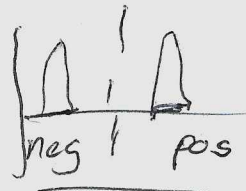
6 tubes



3 Million cells

300K ~ 10 tubes
200K ~ 15 tubes

Violet Red Unstained
Sc's CD3 F.s



3 million cells / mL

200K cells //

$$2/3.0 = 67.1$$

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Sc's 1st run @ 3:11pm
2nd run @ 3:

FS Fix C 3:40 → 50 → 00 → 10 ✓ ✓ ✓
1st Wash @ 4:12

Sc mtra @ 3:45pm → 4:25pm 3rd wash @ 4:28

2nd Wash @ 4:19

4th wash @ 4:35

FS Intra @ 4:27pm →
5:07pm

How to ~~XX~~
Handle evening QC update
parsing ~~XX~~

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BV421

BV510

BV605

BV650

F1c

PerCP-eflor700

PE

PE-Duzzle 594

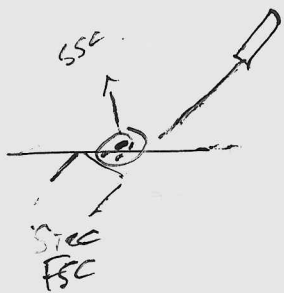
Pe-Cy7

Pe-Vio 770

APC

AF647

APC-Fire 750



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97A
9437A
0250-125A

97
9437
0250-125A

0250-125A

97
9437
0250-125A

97



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αβ T cell SFC panel

#	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 Lys. then FixPerm	Intranuclear Stain 40 min @RT	2
UV1																	
UV2		BUV395	CD62L	(DREG-56)	1							1.2	2.4				
UV6																	
UV7		BUV563	CD69	(FNS0)	2											0.8	1.6
UV9		BUV615	CD69	(1G1)	2					1.8	3.6	0.5	1			0.1	0.2
UV10		BUV661	CD38	(B6)	1							0.5	1			0.7	1.4
UV14		BUV737	CD4	(S13)	1					1.5	3						
UV16		BUV805															
V1																	
V2		Pacific Blue	CD14	(M5E2)	2							1.5	3				
V3		Pacific Blue	CD19	(H1B19)								1.5	3				
V4																	
V5		BUV480	CD161	(REA631)	1					1.5	3			Strep <0.4>			
V6																	
V7																	
V8		BUV570	CD16		1							0.9	1.8				
V9		BUV605	CD45RA	(J252D4)	1							1.0	2				
V10		BUV650	CD8		1							1.0	2				
V13		BUV711	Var/2		2							1.2	2.4				
V14		BUV750	IFNγ	(B27)	1					1.2	2.4					1.5	3
V15		BUV786	CCR6	(11A9)	1												
V16		Alexa Fluor 488	FoxP3														
B2		Spark Blue 550	CD3	(SK7)	1							1.2	2.4			3	6
B3		Spark Blue 574	CD45		1							1.2	2.4			0.5	1
B4		RB613	PD1		1					1	2	0.6	1.2				
B6																	
B7																	
B8																	
B9		RB705	CD26?	(M-A261)	1							<1>				2	4
B10																	
B12																	
B13		RB780	CXCR5?		2												
B14		PE	ICOS	(EH12.2H7)	1					<0.05>							
YG1																	
YG2		PE-Dazzle 594	TNFr	(MAB11)	2											1	2
YG3																	
YG4		PE-Cys	CXCR3		2					1	2						
YG5																	
YG6		PE-Fire 700	CD127		1							1.5	3				
YG7		PE-Fire 744	CD25	(L243)	2					1.2	2.4	0.6	1.2				
YG8		PE-Vio 770	HLA-DR		1												
YG9		PE-Fire810	CD39	(A1)	1					1.2	2.4					1.2	2.4
YG10		APC		(MO1-)	2												
R1		Alexa Fluor 647															
R2																	
R3		APC-R700	CD107a	(H4A3)	1.2	2.4											
R4																	
R5		Zombie NIR	L/D		2												
R6		APC-Fire 750	CD27	(O323)	1							1.5	3				
R7		APC-Fire 810	CGR7														
R8																	
And UNSTAINED CONTROLS !!!																	
Antibody Total					1.2												
R10 Media					19.3												
Pipette draw volume					19.5												
Antibody Total					11.4					1	2	14.7	29.4			10.8	21.6
Brilliant Stain					22.8							29.4	58.8			21.6	43.2
Pipette draw					31.2							41.1				29.4	

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

Project	Location	Area (sq. ft.)	Volume (cu. ft.)	Weight (lb.)	Notes
1	1000	1000	1000	1000	
2	2000	2000	2000	2000	
3	3000	3000	3000	3000	
4	4000	4000	4000	4000	
5	5000	5000	5000	5000	
6	6000	6000	6000	6000	
7	7000	7000	7000	7000	
8	8000	8000	8000	8000	
9	9000	9000	9000	9000	
10	10000	10000	10000	10000	
11	11000	11000	11000	11000	
12	12000	12000	12000	12000	
13	13000	13000	13000	13000	
14	14000	14000	14000	14000	
15	15000	15000	15000	15000	
16	16000	16000	16000	16000	
17	17000	17000	17000	17000	
18	18000	18000	18000	18000	
19	19000	19000	19000	19000	
20	20000	20000	20000	20000	
21	21000	21000	21000	21000	
22	22000	22000	22000	22000	
23	23000	23000	23000	23000	
24	24000	24000	24000	24000	
25	25000	25000	25000	25000	
26	26000	26000	26000	26000	
27	27000	27000	27000	27000	
28	28000	28000	28000	28000	
29	29000	29000	29000	29000	
30	30000	30000	30000	30000	
31	31000	31000	31000	31000	
32	32000	32000	32000	32000	
33	33000	33000	33000	33000	
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35	35000	35000	35000	35000	
36	36000	36000	36000	36000	
37	37000	37000	37000	37000	
38	38000	38000	38000	38000	
39	39000	39000	39000	39000	
40	40000	40000	40000	40000	
41	41000	41000	41000	41000	
42	42000	42000	42000	42000	
43	43000	43000	43000	43000	
44	44000	44000	44000	44000	
45	45000	45000	45000	45000	
46	46000	46000	46000	46000	
47	47000	47000	47000	47000	
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99	99000	99000	99000	99000	
100	100000	100000	100000	100000	

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