

October 26th, 2024

PDI, CD25, CD39, Icas
& the 2 StarBright Dyes

36,

Specimen	Status	Location	Conc	Date	Notes	Volume	CD45+	Total	Resuspension
NY070	Adult		15EG	4/11/24		1	5L 15.0E+4	15EG	↑ 4 mL
NY072	Adult		15EG			1.82	hercept 6.8*10 ⁶	12.2EG	↑ 4.3 ml

Friday 25th October

5:30 AM Thaw NY070

5:34 spin

Twisted CO₂ knob small line
to restore 5%

5:47 am DNase

5:56 am stan count

6:23 am Incubator Rest start

1:31 pm SEB (5pg) added

1:37 pm CD107a (12pl) in

3:47 pm Golgi Block added

Saturday 26th October

6:55 pm spin down (15+2 hrs)

8:06 am Abs prepped

8:59 am Reagents prepped

9:32 am Se spin down

9:47 am Se stains

9:56 am Hot Se → 10:26 am

10:04 am cold Se → 10:34 am } Spec @ 10:35

10:07 am L/P → 10:22 am spin @ 10:26 am

10:56 am Hot samples → 11:26 am spin @ 12:23

11:03 Se's Fix → 13 → 23 → 33 4th wash
including BV 480 ") spin @ 37

11:41 am cold Se's → 12:11 pm

12:11 Se's intrac → 12:51 pm

12:16 pm cold wash main f.s, accidental 0.4% PFA
vs RBC lysis for 3 specimens; immediate
FBS wash, skipping RBC lysis at this point.

	old	new	ctrl	
Med. a	○	○	○/○	CD4 test
SEB	⊗	⊗	○/○	CD39
			○/○	Icas

(Luckerna builds successfully w/o IT firewall
basilist functions are deleted)

★ CD4 samples got sample 1/2 doses
due to Ab sheet screw up ★

Start @ 12:26 → 12:41 pm
Spin @ 12:44 pm
Samples fix @ 12:54 → 1:04 → 1:14 → 1:24
Intrac Se's @ 12:56
2nd @ 1:04 pm
Samples 1st person @ 1:26 pm
Intrac 2nd @ 1:35 pm
1:46 pm → 2:26 pm
3rd 2:30 wash
4th 2:40 final wash
resuspension w/ 40pl of 0.4% PFA-RBC
Done @ 2:50 pm PM

October 26th, 2024

SBB810 bright B14 ^{10¹⁵}

Jcos all + for @ this point?

SBB720 dim ~ RB6B

Lots of n.s. junk

★ Apparently enough for SCS?

Done Acquisition @ 8:31pm
& shutdown

October 26th, 2024

#	Filter	Fluorochrome	Marker	Clone	Dose	During stim!!!	10	L/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	10	Cold Stain 30min @4C	10	4°C for 15 min	then RBC lysis	Intracellular Stain 40 min @RT	10
1	UV7	BUV395	CD62L	(DREG-56)	1							1.2	12				
2	UV9	BUV563	CD69	(FN50)	2												
3	UV10	BUV615	CD34	(1G1)	2					1.8	18	0.5	5			0.8	8
4	UV11	BUV661	CD38	(B6)	2							0.5	5			0.1	1
5	UV14	BUV737	CD38	(SK3)	1							0.5	5				
6	UV16	BUV805	CD4	(SK3)	1					1.5	15					0.7	7
7	V1	BVA421	CD127/CD39	(N5E2)	1					1.5							
8	V3	Pacific Blue	CD14	(H1B19)	2							1.5	15				
9	V5	BVA480	CD161	(REA631)	1					1.5	15	1.5	15				
10	V7	BV510	CD45RA	(H1100)	1							0.5	5				
11	V8	BV570	CD45RA	(H1100)	1									Strep			
12	V10	BV605	CD45RA	(1252D4)	2					2.5	25						
13	V13	BV711	CD72	(B27)	2							1.2	12			1.5	15
14	V14	BV750	CD72	(B27)	2												
15	V15	BV786	CD86	(11A9)	1					1.2	12						
16	B2	Alexa Fluor 488	CD3	(SK7)	1							1.2	12			3	30
17	B3	Spark Blue 550	CD3	(SK7)	1							1.2	12			0.5	5
18	B4	Spark Blue 574	CD8	(SK7)	1							1.0	10				
19	B6	RB613	CD8	(SK7)	1												
20	B9	PerCP-Cy5.5	CD26	(M-A261)	1					1						2	20
21	B14	StarBright B810	CD4	(M-A261)	1												
22	B14	StarBright B810	CD4	(M-A261)	1												
23	B14	StarBright B810	CD4	(M-A261)	1												
24	B14	StarBright B810	CD4	(M-A261)	1												
25	B14	StarBright B810	CD4	(M-A261)	1												
26	B14	StarBright B810	CD4	(M-A261)	1												
27	B14	StarBright B810	CD4	(M-A261)	1												
28	B14	StarBright B810	CD4	(M-A261)	1												
29	B14	StarBright B810	CD4	(M-A261)	1												
30	B14	StarBright B810	CD4	(M-A261)	1												
31	B14	StarBright B810	CD4	(M-A261)	1												
32	B14	StarBright B810	CD4	(M-A261)	1												
33	B14	StarBright B810	CD4	(M-A261)	1												
34	B14	StarBright B810	CD4	(M-A261)	1												
35	B14	StarBright B810	CD4	(M-A261)	1												
36	B14	StarBright B810	CD4	(M-A261)	1												
37	B14	StarBright B810	CD4	(M-A261)	1												
38	B14	StarBright B810	CD4	(M-A261)	1												
39	B14	StarBright B810	CD4	(M-A261)	1												
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41	B14	StarBright B810	CD4	(M-A261)	1												
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94	B14	StarBright B810	CD4	(M-A261)	1												
95	B14	StarBright B810	CD4	(M-A261)	1												
96	B14	StarBright B810	CD4	(M-A261)	1												
97	B14	StarBright B810	CD4	(M-A261)	1												
98	B14	StarBright B810	CD4	(M-A261)	1												
99	B14	StarBright B810	CD4	(M-A261)	1												
100	B14	StarBright B810	CD4	(M-A261)	1												

αβ T cell SFC panel

CD4⁺ T cell SFC panel

CD4⁺ T cell SFC panel

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

Add ColdStain mix, incubate @ 4C for 30min
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min

Add 300-500 ul 1x RBC Lysis for 3 minutes
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min

Add Streptavidin Mtx, 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 PermWash: 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 PermWash: 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

+3 unstained cells
+1 unstained beads

Wash

reason each CD4 sample got 20p1 vs 46p1

5 sc's * 4 * 750p1 = 15
34 sc's * 2 * 750p1 = 51
10 samples * 4 * 1.5 ml = 60
126 mls

39 sc's * 460p1

10 samples * 800p1

Fix
156
8 ml

23.6 ml

5.9/17.7 ml

39 * .08 = 3.12
800

4.120 ml PFA-PBS

3 ml RBC lysis

.3 + 2.7 ml

39 * .750 = 29.25
10 * 6 = 60.00
89.25 [FBS-PBS]

8.8 ml Zombie NIR mix
4 = 10 ml

44
10

1000 1000 1000 1000
1000 1000 1000 1000

1000 1000 1000 1000

1

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