September 27th, 2024

Va7.2/CD27 locations + bonus PD1 RB613 test

Specimen	Status	Location	Conc	Date	Notes	Volume	CD45+	Total	Resuspension
NY071	Adult		15E6	4-11-23		2	~~9.2~~ E6	14.6	↑4.7 total
NY072	HC Leftover Adult yesterday					1	9.9E6	9.9	↑ 3.3 total

Friday

Start @ 9:32 AM
DNAse @ 9:53 AM
StainCond @ 10:01
TrafficJam Auroras
To Rest Suspension @ 10:22 pm
Antigens start @ 4:22 pm
Antigens/CD107a added @ 4:49 pm → 6:49 pm
Golgi @ 6:54 pm → 6:54 am

NY071
ctl OO ctl OOO
SEB O SEB O

NY072
ctl O ctl O
SEB O SEB O

11x10 = 110 µL

6:6:114 µL

Bref Monensin: R10

Saturday

Badge access issues
Arrived @ 6:58 am
Sample spin 7:06 am
SC spin 7:23 am
Ab prep @ 7:44 am
Rinse 8:16 am
Reagents @ 8:31 am

$95

Start @ 8:57 am

WD @ 9:03 am

Cold sc's @ 9:15 am → 45 spin @ 9:47 am
Hot sc's @ 9:26 am → 56 spin @ 9:58 am
Samples Hot @ 9:49 am → 10:11 spin @ 10:14 am
SC's Fix @ 10:11 → 21 → 31 → 41 spin 1° @ 10:46 am
Samples cold @ 10:23 am → 10:53 am

11:03 AM ~~Extra~~ 2nd Perm Wash for sc's
11:12 AM strept cold RBC wash for samples
11:21 Wash sc's away
11:24 Intra sc's → 12:04 pm (samples)
11:29 am strep wash
spin 12:05 pm

11:38 am Samples Fix
→ 48 → 58 → 12:08 pm
12:12 pm Final sc wash & 1st sample Perm wash
12:21 pm sc's done, resuspended in 80 µl 0.4% PFA-PBS
Intrac Samples @ 12:31 pm
3rd Perm wash @ 1:11 pm
Done @ 1:30 pm

Acquisition @ 4:50 (after warmup/QC starting around 4 pm)
ctrl sc's ~~2300~~ cells/sec
BV650 A6 ∉ working (or too little)

RB613 seems dim
Is everything okCR3+?
Horrid AF profile mostly dead
old cells ∉ culture

September 27th, 2024

SEB units 10 ← 1ml = 100µL

ctrl units 36 ← 3ml = ↑ 600µL
2 then 100µL

2 SCs beads + 1 unst = 3

30 SCS cells + 4 unst
− 5 intrac
25 + 4 = 29

5 intrac

5 samples × 4 × 1.5 = 30ml

37 scs × 0.75 = 27.5 ml

7.5ml FBS-PBS

300
× 5
1.5 ml

1.35 ml H_2O
+ 150µL RBC lysc

5 × 0.8 = 4.0

37 × 0.4 = 14.8

18.8 mls
4.7 mls Fix
14.1 mls Dilucit

5 × 1.5 × 4 = 30.0

29 × 0.75 × 2 = 21.75

5 × 0.75 × 4 = 15.00

66.75

60.08 mls H_2O
+ 6.67 mls PermWash

6 × 8 = 5.8 mL
PBS
+ 2µL
Zombi

Samples got all differentials @ the hot stage (Sc's prepped @ respective temps)

421 127 / 711 Va7.2 ○ ○ ○ ← 421 CD127 / 711 Va7.2 / RB613 PD1

September 27th, 2024

αβ T cell SFC panel

#	Filter	Fluorochrome	Marker	Clone	Dose	During stim!!!	5	L/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	5	ColdStain 30min @4C	5	4*C for 15 min	RBC Lyse, then Fix/Perm	Intranuclear Stain 40 min @RT	5
1	UV2	BUV395	CD62L	(DREG-56)	1							1.2	6				
	UV7	BUV496															
2	UV9	BUV563	CD69	(FN50)	2											0.8	4
3	UV10	BUV615	CCR4	(1G1)	2					1.8	9						
4	UV11	BUV661	Vδ2	(B6)	2							0.5	2.5			0.1	0.5
5	UV14	BUV737	CD38		1							0.5	2.5				
6	UV16	BUV805	CD4	(SK3)	1					1.5	7.5					0.7	3.5
7	V1	BV421	Va7.2/CD127		1or2					1.5 (127)		1.2 (7.2)					
8	V3	Pacific Blue	CD14	(M5E2)	2							1.5	7.5				
	V3	Pacific Blue	CD19	(HIB19)								1.5	7.5				
9	V5	BV480	CD161	(REA631)	1					1.5	7.5			Strep ~~<0.8>~~ 0.4			
10	V7	BV510	CD45RA	(Hi100)	1							0.5	2.5				
	V8	BV570															
11	V10	BV605	CXCR5	(J252D4)	2					2.5	12.5						
12	V11	BV650	CD127		1							1.0					
13	V13	BV711	Va7.2		1							1.2					
14	V14	BV750	IFNγ	(B27)	2											1.5	7.5
15	V15	BV786	CCR6	(11A9)	1					1.2	6						
16	B2	Alexa Fluor 488	FoxP3		Beads											3	15
17	B3	Spark Blue 550	CD3	(SK7)	1							1.2	6			0.5	2.5
18	B4	Spark Blue 574	CD8		1							1.0	5				
19	B6	RB613	PD1		1							1.0					
20	B9	PerCP-Cy5.5	CD26	(M-A261)	1											1.8	9
21	YG1	PE	PD1	(EH12.2H7	1							1.0					
22	YG3	PE-Dazzle 594	TNFα	(MAB11)	2											1	5
23	YG5	PE-Cy5	CXCR3		2					1	5						
24	YG8	PE-Fire 744	CD25		2					1.2	6						
25	YG9	PE-Vio 770	HLA-DR	(L243)	1							0.6	3				
	YG10	PE-Fire810															
26	R1	APC	CD39	(A1)	1					1.2	6						
27	R2	Alexa Fluor 647	IL2	(MQ1-	2											1.2	6
28	R4	APC-R700	CD107a	(H4A3)	Supernatan	1.2	6										
29	R6	Zombie NIR	L/D		2			<1:2500>									
30	R7	APC-Fire 750	CD27	(O323)	1							1.5	7.5				
31	R8	APC-Fire 810	CCR7		1					1	5						
And UNSTAINED CONTROLS !!!				Antibody Total	1.2			Antibody Total		12.9	64.5	14.2	71.0			10.6	53
				R10 Media	19.3			Brilliant Stain		25.8	129	28.4	142			21.2	106
				Pippette draw volume	19.5			Pippette draw		35.7		39.6				28.8	

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-PBS
Cap tubes, wrap rack in foil, store at 4*C

September 27th, 2024

Good thing I quarantined off the dd cells from my sc's, they are mostly dead or off AF!

Aggregate sig Σ exp level matter $\propto$ fluorescent photon efficiency

N4022 the old samples $\neq$ dead? vs. $\doteq$ unstained? (washes?)

Shutdown OC @ 6:16 pm