

mod se as norm...

#	Filter	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	2	U/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	2	Cold Stain 30min @4C	2	4°C for 15 min	ReC Lyse, then FixPerm	Intranuclear Stain 40 min @RT	2
1	UV2	BUV395	CD62L	(DREG-56)													
2	UV7	BUV496	CD8	(RPA-T8)													
3	UV9	BUV563	CD69	(FN50)													
4	UV10	BUV615	CD4	(1G1)													
5	UV11	BUV661	Vβ2	(B6)													
6	UV14	BUV737	CD38														
7	V1	BUV805	CD4	(SK3)													
8	V3	Pacific Blue	Vα7.2	(M5E2)													
9	V5	BUV480	CD161	(H1B19)													
10	V7	BUV510	CD45RA	(REA631)													
11	V10	BUV605	CD45RA	(H1B19)													
12	V13	BUV711	ICOS	(H1B19)													
13	V14	BUV750	IFNγ	(B27)													
14	V15	BUV786	CCR6	(11A9)													
15	B2	Alexa Fluor 488	FoxP3														
16	B3	Spark Blue 550	CD3	(SK7)													
17	B9	PerCP-Cy5.5	CD26	(M-A261)													
18	YG1	PE	PD1	(EH12.2H7)													
19	YG3	PE-Dazzle594	TNFR	(MAB11)													
20	YG5	PE-Cy5	CD25	(M-A251)													
21	YG8	cFluor-BYG750															
22	YG10	PE-vio770	H1A-DR	(L243)													
23	R1	APC	CCR7	(A1)													
24	R2	Alexa Fluor 647	IL2	(M01-1)													
25	R4	APC-R700	CD107a	(H4A3)													
26	R6	Zombie NIR	L/D														
27	R7	APC/Fire 750	CD27	(O323)													
28	R8	APC/Fire 810															
And UNSTAINED CONTROLS !!!										Antibody Total	1.5	Antibody Total	10.9	21.8	13.8	27.6	21
										R10 Media	19.0	Brilliant Stain	21.8	43.6	27.6	55.2	42
										Pipette draw volume	19.5	Pipette draw	29.7				

cp T cell SFC panel

Wash with 1 ml PBS, spin down 1300rpm 8min
 800 ul of LiveDead mix (1:2500) @RT for 15min
 Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min
 Add CD161 biotin antibody for 10 minutes at RT
 Add HoStain mix, incubate @ 37C for 30min
 Wash 2 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 2 ml 5% PBS-FBS 1400 rpm, 6min
 Add Streptavidin Mix, incubate @ 4C for 15min
 Wash 2 ml 5% PBS-FBS 1400 rpm, 6min
 1 ml Nuclear FixPerm, incubate @ 4C for 30min
 (vortex every 10 minutes)
 First PermWash: 2 ml NuclearWash 1500 rpm 6 min
 Second Perm Wash: 2 ml NuclearWash 1500 rpm 6 min
 Add Intracellular Stain, incubate @ RT for 40min
 First PermWash: 2 ml NuclearWash 1500 rpm 6 min
 Second Perm Wash: 2 ml NuclearWash 1500 rpm 6 min
 Resuspend in 30 ul 0.4% PFA-PBS
 Cap tubes, wrap rack in foil, store at 4°C

4+1 = intra

28.5cs + 2 und

12ml = 13.6ml = 13.6ml

750
60
45000

2 CKCRS a.fika?

000 Icos... stim?

4...Sigh...

600pl RBC lysis

60pl ~ 540pl

QBC @ 11:03 wash @ 11:27

→ 11:13

Sept @ 11:15 → 11:20

11:32 wash

Fix @ 11:45 → 11:55

1st wash @ 12:21 PM

Intra @ 12:50 PM

SCs done @ 12:55

1:34 PM 3rd spin

1:43 PM 1 wash

7:04 am Abso cell count

7:00 am Samples spin

7:38 am SCs spin

7:47 am Anti bodies spin down

8:33 Ab mix done

Note: Icos + Intra very similar

9:20 AM 2nd spin

9:28 AM 3rd spin

9:36 1st spin

9:48 Samples Hot wash @ 10:23 (+5)

10:30 cold Samples → 11:00 AM

Acquisition @ 3:12
 85 pl ~ 1300 SFC

