

Hand
22nd?

March 7th, 2025

#	Filter	Fluorochrome	Marker	Clone	Dose	During stim!!	3	L/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	Cold Stain 30min @4C	4°C for 15 min	αβ T cell SFC panel	Intracellular Stain 40 min @RT
1	UV1	BUV395	CD62L	(DREG-56)	1						1.2	4.8		
2	UV6													
3	UV9	BUV563	CD69	(F550)	2									
4	UV10	BUV615	CCR4	(1G1)	2					1.8	7.2			0.8
5	UV11	BUV661	Vβ2	(86)	2						0.5	2		0.4
6	UV14	BUV737	CD38	(SK3)	1						0.5	2		0.2
7	UV16	BUV805	CD4	(SK3)	1					1.5	6			0.8
8	V2	Spark Violet 423	CD28	(MSF2)	2						<1.2>	6		
9	V3	Pacific Blue	CD14	(H1B19)	2						1.5	6		
10	V4													
11	V5	BUV480	CD161	(REA631)	1					1.5	6			
12	V6													
13	V7													
14	V8	BUV570	CD16		1						0.9	3.6		
15	V9													
16	V10	BUV605	GD45RA	(1252D4)	1						0.9	3.6		
17	V11	BUV650	GD8		1						0.9	3.6		
18	V13	BUV711	Vα7.2	(827)	2						1.0	4		
19	V14	BUV750	IFNγ	(11A9)	1						1.2	4.8		
20	V15	BUV786	CCR6		1									
21	V16	Alexa Fluor 488	FoxP3							1.2	4.8			1.5
22	B2	Spark Blue 550	CD3	(SK7)	1						1.2	4.8		3
23	B4	Spark Blue 574	CD45		1						0.6	2.4		0.2
24	B6	RB613	PD1		1					1	4			0.8
25	B7													
26	B8													
27	B9													
28	B10	RB705	GD26	(M-A261)	1						1.0	4		
29	B12													
30	B13	RB780	CKR5		2						1.2	4.8		<0.2>
31	B14	PE	ICOS	(EH12.2H7)	1									1.5
32	YG1	PE-Dazzle 594	TNFr	(MAB1)	2									1
33	YG2													
34	YG3	PE-Cy5	CKR3		2					1	4			4
35	YG4													
36	YG5	PE-Fire 700	CD127		1						1.5	6		
37	YG6	PE-Fire 744	CD25	(12A3)	2					1.2	4.8			
38	YG7	PE-Vio 770	HLA-DR	(CD56)	1						0.6	2.4		
39	YG8	APC	CD39	(A1)	1						<1.5>			
40	R1	Alexa Fluor 647	IL2	(MO1-2)	1					1.2	4.8			
41	R2													
42	R3	APC-R700	CD107a	(H4A3)	1.2	3.6								1.2
43	R4													4.8
44	R5	Zombie NIR	L/D		2									
45	R6	APC-Fire 750	CD27	(O323)	1						<1.2500>			
46	R7	APC-Fire 810	CD27		1									
47	R8													
48	And UNSTAINED CONTROLS !!!													
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23/1/2025

16/4/2025

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 4C

$\alpha\beta$ T cell SFC panel

Wash with 2 ml PBS, spin down (3000 rpm 8 min)
 800 μ l of LiveDead mix (1:2500) @RT for 15 min
 Wash 1.5 ml 5% PBS-FBS, spin 1300 rpm, 8 min

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 CoStain mix, incubate @ 4C for 30min
 Add 300-500 μ l 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 μ l 0.4% PFA+BS
 cap tubes, wrap rack in foil, store at 4°C

