

July 29th, 2025

αβ T cell SFC panel

#	Filter	Fluorochrome	Marker	Clone	Dose	During stim!!!	24	L/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	24	ColdStain 30min @4C	24	4°C for 15 min	RBC Lyse, then Fix/Perm	Intranuclear Stain 40 min @RT	24
1	UV1	Spark UV 387															
	UV2	BUV395	CD62L	(DREG-56)		1						1.2	28.8				
	UV6	chluor UV440															
	UV7	BUV495															
2	UV9	BUV563	CD69	(FN50)	2											0.8	19.2
3	UV10	BUV615	CCR4	(1G1)		2				1.8	43.2						
4	UV11	BUV661	Vδ2	(B6)		2						0.5	12			0.1	2.4
5	UV14	BUV737	CD38			1						0.5	12				
6	UV16	BUV805	CD4	(SK3)		1				1.5	36					0.2	4.8
	V1	BY421/															
	V2	Spark Violet 423															
7	V3	Pacific Blue	CD14	(M5E2)		2						1.5	36				
	V3	Pacific Blue	CD19	(HIB19)								1.5	36				
	V4	SYTOX Blue															
8	V5	BV480	CD161	(REA631)		1				1.5	36			Strep <0.4>			
	V6	SBV515															
	V7	BV510															
9	V8	BV570	CD16			1						0.8	19.2				
	V9	Orbital 545															
10	V10	BV605	CD45RA	(J252D4)		1						0.9	21.6				
11	V11	BV650	CD8			1						1.0	24				
12	V13	BV711	Va7.2			2						1.2	28.8				
13	V14	BV750	IFNγ	(B27)	2											1.5	36
14	V15	BV786	CCR6	(11A9)		1				1.2	28.8						
	V16	L/D NIR 786															
15	B2	Alexa Fluor 488	FoxP3			Beads										3	72
16	B3	Spark Blue 550	CD3	(SK7)		1						1.2	28.8			0.2	4.8
17	B4	Spark Blue 574	CD45			1						0.6	14.4				
18	B6	RB613	PD1			1				1	24						
	B7	NovaFluorBlue660															
	B8	SB6675															
	B9	NovaFluorBlue690															
19	B10	RB705	CD26	(M-A261)		1						1.0	24				
	B12	RB744/															
	B13	NovaFluorBlue760															
20	B14	RB780	CXCR5			2						1.2	28.8				
21	YG1	PE	ICOS	(EH12.2H7)	1											1.5	1.5
	YG2	Spark 595															
22	YG3	PE-Dazzle 594	TNFα	(MAB11)	2											0.9	21.6
	YG4	Spark 607															
23	YG5	PE-Cy5	CXCR3			2				1	24						
	YG6	NovaFluorYellow690															
24	YG7	PE-Fire 700	CD127			1						1.5	36				
25	YG8	PE-Fire 744	CD25			2				1.2	28.8						
26	YG9	PE-Vio 770	HLA-DR	(L243)		1						0.6	14.4				
	YG10	PE-Fire810															
27	R1	APC	CD39	(A1)		1				1.2	28.8						
28	R2	Alexa Fluor 647	IL2	(MQ1-	2											1.2	28.8
	R3	cFluor 685															
29	R4	APC-R700	CD107a	(H4A3)	Supernatant	1.2	28.8										
	R5	NovaFluorRed725															
30	R6	Zombie NIR	L/D			2		<1:2500>									
31	R7	APC-Fire 750	CD27	(O323)		1						1.5	36				
32	R8	APC-Fire 810	CCR7			1				1	24						
Antibody Total						1.2		Antibody Total		11.4	273.6	16.7	400.8			9.4	225.6
R10 Media						19.3		Brilliant Stain		22.8	547.2	33.4	801.6			18.8	451.2
Pippette draw volume						19.5		Pippette draw		31.2		47.1				25.2	

Wash with 1 ml PBS, spin down 1300rpm 8min
750 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

0.75 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