

B14 for PF-Fre 810

JMc AIM Panel - NY062 - Ture SEB for 6 hrs - 12/02/22

er 23rd 2022 MADI T cell SFC panel - AIM

Filter	Single color (ul)	Ref cth type	Unmixing cth name	Fluorochrome	Marker	Clone	Vial lot #	During stimuli	1	L/D 15 min RT	37°C for 30min	6	Surface 4°C for 30min	6	4°C for 15 min	RBC then CytoF/P erm	Intra MKRT for 40min	6	6
UV2				BUV395	CD62L	(DREG-56)							1.2	7.2					
				AF	AF-UV6	(RPA-T8)							0.7	4.2			0.25	1.5	
UV7				BUV496	CD8	(FM50)							0.5	3			0.5	3	
UV9				BUV563	CD69	(1G1)													
UV10				BUV615	CCR4														
UV11				BUV661															
UV11				BUV737	CD63	(1C6/CCR3)					3.0	18					0.25	1.5	
UV11				BUV805	CD4	(S3)							1.5	9					
UV16				BUV421	CD127	(A019D5)							2.0	12					
V1				Pacific Blue	CD14	(M5E2)							2.0	12					
V3				Pacific Blue	CD19	(H1B19)							0.7	4.2					
V3				BUV480															
V5				BUV510	CD45RA	(H1100)													
V7				BUV570															
V10				BUV605	CCR5	(1252D4)					2.5	15							
V11				BUV650	CCR7	(6043H7)					1.5	0		0				0	
V13				BUV711	ICOS	(DX29)													
V14				BUV750	CCR6	(827)					1.5	9							
V15				BUV786		(11A9)													
B2				FITC	FoxP3	(236A/E7)							1.2	7.2			0.25	1.5	
B3				Spark blue 550	CD3	(SK7)							1.2	7.2			0.25	1.5	
B4				PE	PD-1	(EH12.2H7)							1.2	7.2			0.25	1.5	
B6				PE-CF594	CD26	(M-A261)							1.2	7.2			0.25	1.5	
B8				PE-Cy5	CD25	(M-A251)							1.2	7.2			0.25	1.5	
B10				PerCP-Cy5.5	CD40L	(24-31)							1.2	7.2			0.25	1.5	
B13				PE-Cy7	CD40L	(RE4656)							1.2	7.2			0.25	1.5	
R1				APC	CD39	(A1)													
R2				AF647															
R2				APC-FITC	CD107a	(H4A3)					6.0	6							
R4				Zombie NIR	Viability														
R6				APC-FITC	CD11b	(8060DEC17)													
R7				APC-FITC	CD11b	(HIT2)													
R8				APC-FITC	CD38														

ICOS, CD40L, OX40, 4-1BB to be added 1.2 ml/sample at 4C

APC 200

Simplified Protocol

- Rest cells overnight in 6x
- Collect, count, aliquot cell
- Add antigens, stim antib
- Cap and store incubator a
- Add golgi block, and incu
- Wash with 1.2 ml PBS, 1:
- 800 ul Live/Dead 1:2500
- Wash 2 ml 5% PBS-FBS 1
- Add hot Surface mix, incu
- Wash 2 ml 5% PBS-FBS 1
- Add cold Surface mix, inc
- Add 300-500ul 1x RBC l
- Wash 2 ml 5% PBS-FBS 1
- 300 ul CytoF/P, perm, incu
- First PermWash:
- Second Perm Wash:
- Add Intracellular Stain, in
- Wash once w/ 2ml Perm
- [Fix for FoxP3, Stain for f
- Resuspend in 100 ul 0.4%
- Cap tubes, wrap rack in fi