

August 4th, 2022

190

Spectral Panel Optimization #3

1:100

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	3E+6
N4062	Adult PBMC		20E6	08/12/ 21	Panel-throwed	4 ml	3.23E6	4.88E6	12.92	928μl	

Stain count @ 01:48

To add test
25 color
hCD1d load
hMRI load

control test
5 unl. hCD1d
5 unl. hMRI

Total colors + Autofluorescence

5K 0: Unstained ctrl - color

AutoFluor

RefLibrary

bright or brighter

NKT

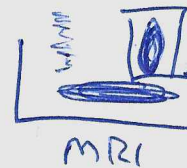
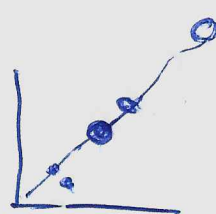
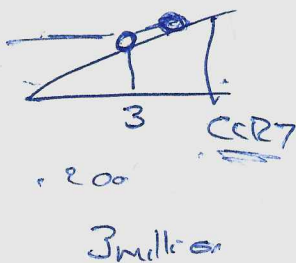
hCD1d

hCD1d

NKT

lipid - hCD1d
PB5-57

CCR7 3M



4:38 creating 1 pre prep 1.5 2.0 f 1

4:39 cells out of incubator

5:04 CCR7 in for stain → 5:10 4/0 at

Tetramers @ 5:29 → 6:10

CCR7 SiH3 in for FixPerm → wash cell @ 6:14

ILT Tetramer tubes into 37°C @ 6:25

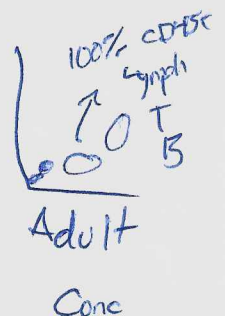
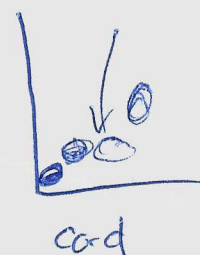
Cold stain @ 7:11

RB lyse @ 7:45pm → wash @ 7:49

FixPerm 7:58

End @ 20:47 pm ✓

red blood cell CD45 -
stem cells CD45 -



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Inmate-like T cell SEC panel

#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial lot #	During stim [min]	L/D 15 min (RT)	Tetramer (RT) for 40 min	37°C (RT) for 30min	2	Surface staining 40°C for 30min	2	lysing then Cytofix/Perm	Intra mixRT for 40min Intra mix	2
1	UV2				BUV395	CD82L	(DRG-56)							1.2	2.4		0.25	0.50
2	UV7				BUV496	CD8	(RPA-178)							0.7	1.4		0.25	0.50
3	UV9				BUV563	CD69	(FN50)							1.0	2.0		0.25	0.50
4	UV10				BUV615	CCR4	(1C1)							0.7	1.4		0.25	0.50
5	UV11				BUV661	V62	(86)							0.7	1.4		0.25	0.50
6	U14				BUV737	CCR3	(1C6/ CCR3)							1.5	3.0		0.25	0.50
7	UV16				BUV805	CD4	(5K3)							1.0	2.0		0.25	0.50
8	V1				BUV421	CD127	(A012D5)							2.0	4.0		0.25	0.50
9	V3				Pacific Blue	CD19	(MSE2)							2.0	4.0		0.25	0.50
10	V5				Pacific Blue	CD19	(H819)							2.0	4.0		0.25	0.50
11	V7				BUV480	CD161	(REA631)							0.7	1.4		0.25	0.50
12	V10				BUV510	CD45RA	(H1D0)							0.7	1.4		0.25	0.50
13	V13				BUV650	CD36								0.7	1.4		0.25	0.50
14	V14				BUV711	CCR7								1.5	3.0		0.25	0.50
15	V15				BUV750	IFN γ	(927)							1.2	2.4		0.25	0.50
16	B2				BUV786	CCR6	(11A9)							1.0	2.0		0.25	0.50
17	B3				Alexafluor 488	hCD1d								1.2	2.4		0.25	0.50
18	B4				Spark blue 550	CD3	(SK7)							1.2	2.4		0.25	0.50
19	B6				PE	NKG2D								1.2	2.4		0.25	0.50
20	B8				PE-CF594	CD26	(M-A261)							1.2	2.4		0.25	0.50
21	B10				PE-CF5	CD25	(M-A251)							1.2	2.4		0.25	0.50
22	B13				PerCP-CF5.5	TNFR	(MAB11)							1.5	3.0		0.25	0.50
23	R1				APC	PD1								1.5	3.0		0.25	0.50
24	R2				Alexa Fluor 647	hMIR1								1.5	3.0		0.25	0.50
25	R4				APC-R700	CD107a	(H4A3)							1.5	3.0		0.25	0.50
26	R6				Zombie NIR	L/D								1.5	3.0		0.25	0.50
27	R7				APC/Fire 750	CD27	(O323)							1.5	3.0		0.25	0.50
28	R8				APC/Fire 810	CD38	(H12)							1.5	3.0		0.25	0.50
And UNSTAINED CONTROLS !!!									8.4	16.8	Antibody Total	12.1	24.2	16.4	32.8	7.25	14.5	
									12.1	24.2	Brilliant Stain	50	100	50.0	100	50	100	
									19.5		Pipette draw volume /sample	61		65.4		56.25		

Simplified Protocol

Aliquot cells 2.0E+6 cells/tube
 Raise R10 + cell volume to 500 ul
 Add 500 ul of PMA-ionomycin/Ctrl
 Incubate 6 hours @37C

Wash with 2 ml PBS, 1400rpm, 6min

800 ul Live/Dead @RT for 15min

Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

Add Tetramers, incubate @RT for 40min

Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

Add hot Surface mix, incubate @37C for 30 min

Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

Add cold Surface mix, incubate @ 4C for 30min

Add 300-500ul 1x RBC lysis for 3 minutes

Wash 2 ml 5% PBS-FBS 1500 rpm, 6min

300 ul Cytofix/perm, incubate @ 4C for 20min

Wash twice 1 ml PermWash

1500rpm, 6min

Add Intra Staining, incubate @ RT for 40min

Wash once w/ 2ml Perm Wash

Resuspend 100 ul 0.4% PFA-PBS, store 4C

PMF
 P450m
 CD307A

