

July 21-22nd, 2022Fresh-thawed PBMCs
Spectral Panel Optimization #2

1:100

Innate-like T cells

2

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	1.5E+6
ND050			15E+6	08/24/21		3 ml	2.23E6	3.44E6	6.7 10.32	1.34 ml	846 µl
ND050			15E+6	08/24/21		3 ml	2.55E6	3.81E6	7.65 11.43	2.4 ml	1.6 ml

ATTN: HOOK for 5% FBS wash
new L-glutamine for media w/ normal FBS, and Gentamycin

The Bioscience flow machine broke!!

Ab prep @ 4:25 pm → 6:48 pm (yikes!!)
starting @ 8 pm

~ 58 sc's

1.2

11.6 + 30 ml

count stain @ 8:30

[2015+1 + 480 → 300 µl]

count @ 8:43 pm

9:25 cells aliquoted

Mod in @ 9:28 pm

37 127 CCR7 102 AM (39:69/8/4 acc in, didn't mess up?)

9:52 pm spin @ 9:58

Abs & tubes redistributed @ 10:31

Tets & Ab broth in @ 22:50

Hot mixes in @ 11:05 pm (full stacks)
→ 35

11:24 pm sc hot ones in

→ 54 pm

spin @ 00:02

no RBC lysis for
Monocytes

Rbolyse MIF @ 11:37 pm

ab/mon in @ 23:41, ILTs out wash same time.

ILTs hot → 00:15 am

11:54 pm / 00:02 ab/mon → cold stain ... @ 11:57 → 12:27 pm
hot sc's out @ 00:12, into drawer

hot ILT out / into hot spin

cold ILT in @ 00:28, cold ab/mon at simultaneously

Rb lysis @ 00:31

ab/mon wash @ 00:36

Ab streak @ 00:49 → 1:05 am

Cold sc's mon + T's in

@ 00:42 → 1:12 am

last cold sc's @ 1:57 →

1:27

ILTs out cold @ 1:58, 1:00 RBC lysis

* Monocytes + hot drawer things stacked

Monocytes done @ 1:51 am → Not doing sc's intracellular smears.

Fix Perm @ 02:00 am @ 11 am vortex

2:46 intercellulars in → 3:26 am

sc's done @ 3:04 am Done @ 3:33 am



#	Filter	Single color (U)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial lot #	During stim III	1	L/D 15 min (RT)	Tetramer (RT) for 40 min	37°C (RT) for 30 min	1	Surface staining 4°C for 30 min	1	lysing, then CytoFk/Perm	Intra mix RT for 40 min	1
1	UV2				BUV395	CD62L	(DfEG-56)								1.2	1.2		0.25	0.25
2	UV7				BUV496	CD8	(RPA18)								0.7	0.7		0.25	0.25
3	UV9				BUV563	CD69	(EN50)								1.0	1.0		0.25	0.25
4	UV10				BUV615	CCR4	(1G1)								0.7	0.7		0.25	0.25
5	UV11				BUV661	V82	(B6)								0.7	0.7		0.25	0.25
6	UV14				BUV737	CD38	(1G6/)								0.7	0.7		0.25	0.25
7	UV16				BUV805	CD4	(S33)								1.5	1.5		0.25	0.25
8	V1				BUV421	CD127	(M01905)								1.0	1.0		0.25	0.25
9	V3				Pacific Blue	CD14	(M5E2)								2.0	2.0		0.25	0.25
10	V5				Pacific Blue	CD19	(H1B19)								2.0	2.0		0.25	0.25
11	V7				BUV480	CD161	(HEA631)								0.7	0.7		0.25	0.25
12	V10				BUV510	CD45RA	(H1100)								0.7	0.7		0.25	0.25
13	V13				BUV605	CD56									0.7	0.7		0.25	0.25
14	V14				BUV711	CCR7									1.0	1.0		0.25	0.25
15	V15				BUV750	IFN γ	(B27)								1.5	1.5		0.25	0.25
16	B2				AlexaFluor 488	hCD1d									1.2	1.2		0.25	0.25
17	B3				Spark blue 550	CD3	(SK7)								1.2	1.2		0.25	0.25
18	B4				PE	NKG2D									1.2	1.2		0.25	0.25
19	B6				PE-CF594	CD26	(M-A261)								1.2	1.2		0.25	0.25
20	B8				PE-Cy5	CD25	(M-A251)								1.2	1.2		0.25	0.25
21	B10				PerCP-Cy5.5	TNFR α	(MAB11)								1.2	1.2		0.25	0.25
22	B13				PE-Cy7	PD1									1.5	1.5		0.25	0.25
23	R1				APC										1.5	1.5		0.25	0.25
24	R2				APC										1.5	1.5		0.25	0.25
25	R4				APC-R700	CD107a	(HMA3)								1.5	1.5		0.25	0.25
26	R6				Zombie NIR	L/D									1.5	1.5		0.25	0.25
27	R7				APC/Fire 750	CD27	(O323)								1.5	1.5		0.25	0.25
28	R8				APC/Fire 810	CD38	(H17)								1.5	1.5		0.25	0.25
And UNSTAINED CONTROLS III																			
R10 Media																			
Pipette draw volume /sample									19.5						61				
Antibody Total									8.4	8.4	Antibody Total	12.1	12.1	12.1	16.4	16.4		7.25	7.25
Brilliant Stain									12.1	12.1	Brilliant Stain	50	50	50	50.0	50		50	50
Pipette draw volume /sample									19.5						61			56.25	

Notes:

Simplified Protocol

Alliquot 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 CytoFk/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

July 21-22nd, 2022

Spectral Panel Optimization #2

Fsc 60 SSC 332 SSC-B 300

I remembered to add (UV7 V7 B3 ~~4~~)
wid the Fsc SSC.

25% unstained cells

Running Full stained ILT @ 6:38 pm

3 400 cells/sec

debris debris field ~~4~~ m

4/m tons @ 2m
(centered)

29
27
26

7/18/2022
























F5c 60 55c 332 55cB-36d
 estimated(A5) 2018 \rightarrow V01 V7 B3 AFS
 up to $5^{A'}$ brightness.
 10²

CD107a

Date		Time		Location		Remarks	
10/10/20	10:00	10:15	10:30	10:45	11:00	11:15	11:30
10/10/20	11:45	12:00	12:15	12:30	12:45	13:00	13:15
10/10/20	13:30	13:45	14:00	14:15	14:30	14:45	15:00
10/10/20	15:15	15:30	15:45	16:00	16:15	16:30	16:45
10/10/20	17:00	17:15	17:30	17:45	18:00	18:15	18:30
10/10/20	18:45	19:00	19:15	19:30	19:45	20:00	20:15
10/10/20	20:30	20:45	21:00	21:15	21:30	21:45	22:00
10/10/20	22:15	22:30	22:45	23:00	23:15	23:30	23:45
10/10/20	24:00	24:15	24:30	24:45	25:00	25:15	25:30
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10/10/20	241:00	241:15	241:30	241:45	242:00	242:15	242:30
10/10/20	242:45	243:00	243:15	243:30	243:45	244:	

#	Filter	Fluorochrome	Marker	Close	Manufacturer	Catalog #	Cell Location	Abundance	Lot #	Control type	Fixed?	Condition	Panel ID	Panel Brightest MFI	Total #	Single color (u)	Negative MFI	Positive MFI	Comments	Reference Library Name
1		BUV395	CD82L	[DEC5-56]												1.5	1			
2		BUV696	CD8	[PPA-78]												1.5	1			
3		BUV653	CD86	[PNA50]												1.5	1			
4		BUV615	CCR4	[IG1]												1.5	1			
5		BUV661	Vβ2	[B6]												1.5	1			
6		BUV737	C/CR3	[1C6/C/CR3]												1.5	1			
7		BUV685	CD4	[8A3]												1.5	1			
8		BUV421	CD127	[AO19D5]												1.5	1			
9		Pacific Blue	CD14	[M5E2]												1.5	1			
10		BUV689	CD161	[BEA53]												1.5	1			
11		BUV510	CD45RA	[H100]												1.5	1			
12		BUV605	CD56													1.5	1			
13		BUV790	IFNγ	[B27]												1.5	1			
14		BUV786	CCR6	[11A9]												1.5	1			
15		AlexaFluor 488	ICD14													1.5	1			
16		Spark blue 550	CD3	[SK7]												1.5	1			
17		PE	NK62D													1.5	1			
18		PE-CF594	CD26	[M-A2A1]												1.5	1			
19		PE-CF5	CD25	[M-A2A1]												1.5	1			
20		PerCP-CF5.5	TRFα	[MA611]												1.5	1			
21		PE-CF6770	PD1													1.5	1			
22		Alexa Fluor 647	hMFI1													1.5	1			
23		APC-CF700	CD107a	[H4A3]												1.5	1			
24		Zombie NIR	1/0													1.5	1			
25		APC/H7e 750	CD27	[O3A3]												1.5	1			
26		APC/H7e 810	CD38	[H172]												1.5	1			
27		BUV650	CCR7													1.5	1			
28																1.5	1			
29																1.5	1			



	BUV395		BUV496		BUV563		BUV615		BUV661		BUV737		BUV805
	BV421		Pacific Blue		BV480		BV510		BV605		BV650		BV750
	BV786		Alexa Fluor® 488		Spark Blue™ 550		PE		PE-Cy5.5		PE-Cy5.5		Alexa Fluor® 647
	PerCP-Cy5.5		PE-Vio® 770		APC-R700		Zombie NIR™		APC-Fire™ 750		APC-Fire 810		

Configuration:4L 16UV-16V-14B-8P

Flexity™ Index: 9.44

July 21-22nd, 2022

Spectral Panel Optimization #2

