David's Copy

Guava Tubes!

May 12-13th, 2022

Spectral Panel Optimization #1

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly 1:100	Ly+Mon	Total	2E+6	1.5E+6
930 pt Inf097-6 2-2	R48N HU	Box 3A I,1				3 ml	3.46E6	5.41E6	10.38	578µl	433µl

HU: Inf197 (complete sets → 9 vials) Box 3A, Row I, Col 1, 2
PM CQ1047 8625 5AE1

9:35 → DNAse I
:45
10:11 cell count start

ILTs PMA ctrl ctrl
○ ○ ○
Full Panel
loaded tetramers

GM

hMR1 - 5OPRU (ag) ✓
GFP (inert)

Vα7.2 mAb

hCD1d ✱ cord blood

hCD1d - PBS-57 (lipid)

ab specific Vα24Jα18 mAb

○ ○ ○ 2M (6)

Unmixing (compensation for)

(for each new

Unstained Controls
○ ○ 300K
PMA ctrl
○ ○

[2] ctrl → 6hr → unstained control 500µl ctrl - PMA

[2] PMA

FITC APC
○ ○ ○
300 300 300
○ ○ ○

2 µl PMA × 2 = 4 µl
800µl (810µl)

2 µl ctrl Golgi × 3 = 6 µl
1200 (1215µl)

2 PMA wells 3 ctrl

578µl - 400µl
cells req'd

2 µl

528 + 72 R10
U 1.8

+ U 400µl R10
+PMA (2) [1]
+ctrl (2) [2]

400 72 528µl

400 72

400 72

1.8

5:51 pm

11:51 AM PMA

200µl U + 300µl R10 U + .77 PMA [1] + .77 golgi [2]
700k → 1.4

Reagents done prepped @ 3:49 pm

250µl 200µl 150µl

.700

1.4 .500 1.8

0.77µl 1.4

100µl

May 12th, 2022 Innate-like T cell SFC panel

2 ml FTS 2 ml FBS 2 ml FBS 2 ml FBS 27 colors

#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	3	L/D 15 min (RT)	Tetramer (RT) for 40 min	37oC (RT) for 30min	3	Surface staining 4oC for 30min	3	Lysing, then CytoFix/ Perm	Intra mixRT for 40min Intra mix	3
1	UV2				BUV395	CD62L	(DREG-56)								1.2	3.6			
2	UV7				BUV496	CD8	(RPA-T8)								0.7	2.1		0.25	0.75
3	UV9				BUV563	CD69	(FN50)								~~1~~ 0.7	3.0		0.25 ~~x 5~~	0.75
4	UV10				BUV615	CCR4	(1G1)						2.0	6					
5	UV11				BUV661	Vδ2	(B6)								0.7	2.1			
6	U14				BUV737	CXCR3	(1C6/ CXCR3)						2.5	7.5					
7	UV16				BUV805	CD4	(SK3)								1.5	4.5		0.25	0.75
8	V1				BV421	CD127	(A019D5)		0.8	2.4					1.0	3.0			
9	V3				Pacific Blue	CD14	(M5E2)								2.0	6.0			
	V3				Pacific Blue	CD19	(HIB19)								2.0	6.0			
10	V5				BV480	CD161	(REA631)		0.8	2.4			1	3				0.5	1.5
11	V7				BV510	CD45RA	(Hi100)								0.7	2.1			
12	V10				BV605	CD56									0.7	2.1			
27	V11				BV650	CCR7			0.8	2.4					1.0	3			
	V13				BV711														
13	V14				BV750	IFNγ	(B27)											1.5	4.5
14	V15				BV786	CCR6	(11A9)						1.8	5.4					
15	B2				AlexaFluor 488	hCD1d						<1:750> (0.2)							
16	B3				Spark blue 550	CD3	(SK7)								1.2	3.6		0.25	0.75
17	B4				PE	NKG2D									1.0	3.0			
18	B6				PE-CF594	CD26	(M-A261)						1.2	3.6					
19	B8				PE-Cy5	CD25	(M-A251)								1.2	3.6		0.25	0.75
20	B10				PerCP-Cy5.5	TNFα	(MAB11)											2.5	7.5
21	B13				PE-vio770	PD1									1.5	4.5			
	R1				APC													~~1.5~~	4.5
22	R2				Alexa Fluor 647	hMR1						0.5 <1:500> (5)							
23	R4				APC-R700	CD107a	(H4A3)		6.0	18									
24	R6				Zombie NIR	L/D					<1:2500>								
25	R7				APC/Fire 750	CD27	(O323)						2	6					
26	R8				APC/Fire 810	CD38	(HIT2)						1.6	4.8					
							Antibody Total		8.4	25.2	Antibody Total		12.1	36.3	16.4	49.2		7.25	21.8
And UNSTAINED CONTROLS !!!							R10 Media		12.1	36.3	Brilliant Stain		50	150	50.0	150		50	150
							Pippette draw volume /sample		19.5		Pippette draw volume /sample		61		65.4			56.25	

Notes:

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

Unstained or beta cell pops

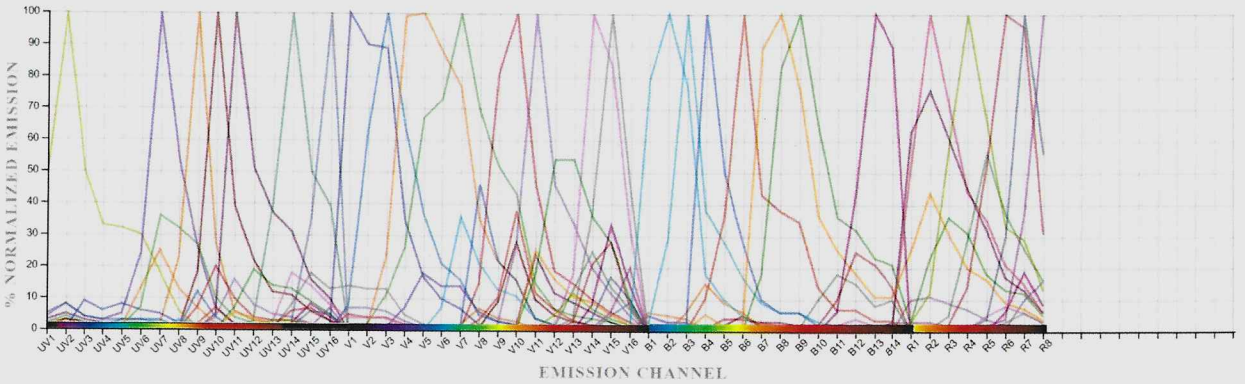
CD3 ~~127~~ ~~56~~

Date: May 12, 2022 — ILT SFC Reference Library

PMA — Ctrl

#	Filter	Fluorochrome	Marker	Clone	Manufacturer	Catalog #	Cell Location	Abundance	Lot #	Control type	Fixed?	Condition	Panel ul	Panel brightest MFI	Total #	Single color (ul)	Negative MFI	Positive MFI	Comments	Reference Library Name
1		BUV395	CD62L	(DREG-56)												1.5 1 0.5				
2		BUV496	CD8	(RPA-T8)												1.5 1 0.5		a		ctrl
3		BUV563	CD69	(FN50)												1.5 1 0.5		ac-v1		2021 b
4		BUV615	CCR4	(1G1)												1.5 1 0.5		a		ctrl
5		BUV661	Vδ2	(B6)												1.5 1 0.5				CCR4
6		BUV737	CXCR3	(1C6/ CXCR3)												1.5 1 0.5		a-VD2-v1		VD2-V1
7		BUV805	CD4	(SK3)												1.5 1 0.5				ctrl
8		BV421	CD127	(A019D5)												1.5 1 0.5		a-CD4-v1		ctrl
9		Pacific Blue	CD14	(M5E2)												1.5 1 0.5				ctrl
10		BV480	CD161	(REA631)												1.5 1 0.5		a-Dump - V1		CBMC
11		BV510	CD45RA	(Hi100)			Surface	Abundant		Cells	Fixed		1µl (:7)			1.5 1 0.5				ac 2
12		BV605	CD56													~~1.5~~ 2 0.5		ac-CD45RA-2		110221
13		BV750	IFNγ	(B27)												1.5 1 0.5		11021		CBMC activated
14		BV786	CCR6	(11A9)												1.5 1 0.5				
15		AlexaFluor 488	hCD1d													1.5 1 0.5				bla24 CD3
16		Spark blue 550	CD3	(SK7)												1.5 1 0.5				CBMC
17		PE	NKG2D													1.5 1 0.5				1121
18		PE-CF594	CD26	(M-A261)												1.5 1 0.5		1121		ctrl
19		PE-Cy5	CD25	(M-A251)												1.5 1 0.5				Ctrl
20		PerCP-Cy5.5	TNFα	(MAB11)												1.5 1 0.5				act
21		PE-vio770	PD1													1.5 1 0.5				1102
22		Alexa Fluor 647	hMR1													1.5 1 0.5		1110		Ctrl
23		APC-R700	CD107a	(H4A3)												1.5 1 0.5				acCBMC
24		Zombie NIR	L/D													1.5 1 0.5				ctrl
25		APC/Fire 750	CD27	(O323)												1.5 1 0.5		V1		ctrl
26		APC/Fire 810	CD38	(HIT2)												1.5 1 0.5		V1		110221
27		BV650	CCR7													1.5 1 0.5		V1		
28																1.5 1 0.5				
29																1.5 1 0.5				

Preliminary ILT Panel



BUV395	BUV496	BUV563	BUV615	BUV661	BUV737	BUV805
BV421	Pacific Blue	BV480	BV510	BV605	BV650	BV750
BV786	Alexa Fluor® 488	Spark Blue™ 550	PE	PE-CF594	PE-Cy™5	Alexa Fluor® 647
PerCP-Cy™5.5	PE-Vio®770	APC-R700	Zombie NIR™	APC-Fire™ 750	APC-Fire 810	

Preliminary ILT panel

Similarity™ Indices

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

	BUV395	BUV496	BUV563	BUV615	BUV661	BUV737	BUV805	BV421	Pacific Blue	BV480	BV510	BV605	BV650	BV750	BV786	Alexa Fluor 488	Spark Blue 550	PE	PE-CF594	PE-Cy5	Alexa Fluor 647	PerCP-Cy5.5	PE-Vio770	APC-R700	Zombie NIR	APC-Fire 750	APC-Fire 810
BUV395	1																										
BUV496	0.25	1																									
BUV563	0.05	0.32	1																								
BUV615	0.03	0.1	0.4	1																							
BUV661	0.04	0.02	0.05	0.32	1																						
BUV737	0.03	0.01	0.02	0.12	0.39	1																					
BUV805	0.11	0.03	0.01	0.05	0.1	0.4	1																				
BV421	0.05	0.09	0.01	0.01	0	0	0.01	1																			
Pacific Blue	0.01	0.13	0.01	0.01	0	0	0	0.78	1																		
BV480	0.02	0.38	0.08	0.05	0.01	0	0	0.28	0.58	1																	
BV510	0.02	0.48	0.24	0.17	0.04	0.02	0	0.17	0.36	0.84	1																
BV605	0.01	0.05	0.14	0.41	0.12	0.05	0.02	0.06	0.07	0.17	0.43	1															
BV650	0	0.02	0.03	0.24	0.37	0.13	0.02	0.1	0.08	0.07	0.16	0.54	1														
BV750	0	0	0	0.04	0.1	0.37	0.14	0.05	0.04	0.02	0.04	0.1	0.24	1													
BV786	0	0.01	0	0.03	0.07	0.29	0.23	0.2	0.15	0.05	0.06	0.09	0.19	0.86	1												
Alexa Fluor 488	0.01	0.06	0.05	0	0	0	0	0	0	0.05	0.01	0	0	0	0	1											
Spark Blue 550	0.02	0.09	0.16	0.06	0.01	0.01	0	0.02	0.05	0.22	0.3	0.17	0.06	0.01	0.02	0.63	1										
PE	0.01	0.05	0.28	0.11	0.01	0	0	0.01	0.02	0.11	0.23	0.27	0.06	0	0	0.14	0.47	1									
PE-CF594	0.01	0.01	0.12	0.19	0.04	0.03	0	0	0.01	0.04	0.11	0.34	0.18	0.02	0.02	0.06	0.28	0.44	1								
PE-Cy5	0	0	0.03	0.07	0.34	0.16	0.02	0	0	0.01	0.02	0.1	0.25	0.09	0.06	0.01	0.12	0.13	0.5	1							
Alexa Fluor 647	0	0	0	0.01	0.71	0.22	0.02	0	0	0	0	0	0.16	0.02	0.01	0	0	0	0	0.38	1						
PerCP-Cy5.5	0	0.01	0.01	0.1	0.33	0.33	0.07	0.01	0.01	0.01	0.02	0.13	0.36	0.31	0.23	0	0.07	0.06	0.38	0.81	0.27	1					
PE-Vio770	0	0	0	0.01	0.03	0.21	0.11	0	0	0	0.01	0.02	0.04	0.25	0.27	0	0.02	0.01	0.05	0.13	0.02	0.27	1				
APC-R700	0	0	0	0.01	0.49	0.46	0.05	0	0	0	0.01	0.02	0.16	0.12	0.07	0	0	0	0.01	0.26	0.64	0.34	0.06	1			
Zombie NIR	0	0.01	0	0.01	0.21	0.53	0.14	0	0	0	0.01	0.02	0.09	0.31	0.27	0	0.01	0	0.02	0.16	0.27	0.26	0.36	0.52	1		
APC-Fire 750	0	0.01	0	0.01	0.13	0.32	0.21	0	0	0.01	0.01	0.01	0.05	0.2	0.23	0	0	0	0	0.08	0.16	0.13	0.21	0.3	0.79	1	
APC-Fire 810	0	0	0	0	0.1	0.21	0.25	0	0	0	0	0.01	0.04	0.12	0.17	0	0	0	0	0.06	0.12	0.09	0.15	0.2	0.43	0.74	1

plexity™ Index: 9.44

May 12-13th, 2022 — Spectral Panel Optimization #1

1:400 8×
1:2000 4×
1:1000 2×

L/D Aqua (1:500) 2:1000 → 1.5 - 2M cells/…

$0.100 50µl → 0.050 * 3.46 = 170K

L/D @ 12:32pm
→ 47pm

1µl L/D : 500µl PBS
2µl : 1000 → 800µl cells 1.5 - 2M
1.6µl → 400 ~~80µl~~ 800µl. 200K
200 ... 500
#100 : (100µl) 200µl 250

500 800µl
60 100
= 720
500 800
200
1:8
1:4

000
3 samples
4 abs

3 ml 5% FBS-PBS Per Sample
3 ml Permwash (water)
8 × 9 = 72ml 5% FBS-PBS
4 × 9 = 36 ml Permwash

10×
RBC lysis
13
300µl × 3 + 10
×
3.900µl 3.000µl
4ml
400µl 10× RBC
3600µl Water

5:49pm centrifuge spin
L/D @ 6:05
Tetramers @ 6:43pm
Hot surface 7:48pm → 8:18pm
Cold 8:42 pm → 9:12 pm
→ 9:17 pm RBC lysis
9:29 pm
1st Permwash @ 9:52
Done c RefLibrary @ 10:16 ✓
Intracellular @ 10:13 → :53
Done @ 11:02 pm

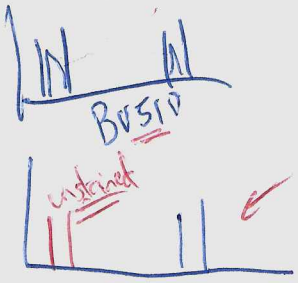
Ref Library ~ 600K — unst 400, 6µl 400, 2µl 400
700K → ~300K ~200K ~200K

17

May 12-13th, 2022

Spectral Panel Optimization #1

UV3 %rCV 11.23



V5

DR-cC-CD45RA-2 ✓ Dr-aUns-fix-051322

aC

(P) 10^{15} gmk ✓ V5J

10^5 V5

Dump ribbed control, PMA-Inc

CD8 slightly off

161 dC

CD27

CD45RA 1.5 µl -> 1p5 Dr-cC-CD45RA-1p5

^5 half full, just feed charge. / for PMA as well

92%

20% CD8

77.7% CD4

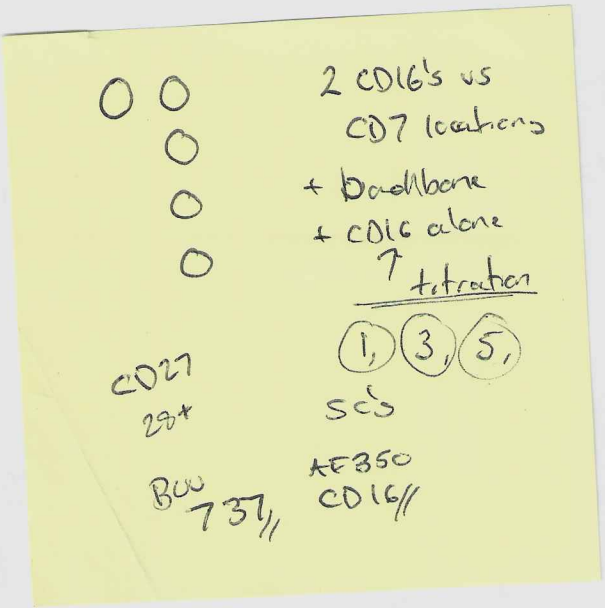
0.8% DN

0.5% Vδ2γδ

0.03% NKT

Pac CD8

CD3 · CD8


2 CD16's vs
CD7 locations
+ backbone
+ CD16 alone
↑ titration
1, 3, 5,
CD27
29+
SCs
BV
737//
AF350
CD16//