



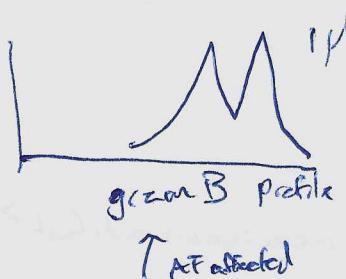
August 17th, 2022

γδ SFC Panels #1

6:14 warm up
6:33 datasheets setup
6:38 start

CH1

15K events for ctrl sc unstained



↑ ACT affected

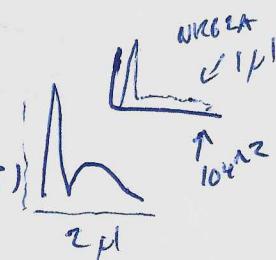
BV11
CD127 low ($\downarrow 10^4$)
Range @ 10^4 1/p

unstained
PBMC-NKG2A-22a

W7 V7 (doesn't look well for CD19?)

ditto perf? @ 10^{14}

(odd pattern few +?)



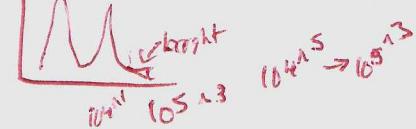
6:53 PMA ↓ Fsc gain toward threshold (shrinking ←, may consider adj up to 70% doublets edge.)

BV421 BV711 low $10^{4.2}$ M 711 higher M xx intensity

NKG2a on APC

donor or amount?
not presentation shrinking cells

APC-Fc150
CD28



[Acquisition @ 7:02 AM]

(stt flush & vortex for cells)

Conc → 1354 cells rate, 21.4 $\mu\text{l}/\text{min}$ ~ 5 min?

Nyosci PMA
Less "shrinking" cells than samples for scs PMA

RBC lysis of samples not of scs? (7:07 → 37/1)

* PMA NKG2A + CD28 37°C carb tube (stt flush twice)
- 2A + 127 BV711 stt flush twice

? too much stan issue?

PMA BV421 all has a higher events rate 1500f-3000

gross can be right or wrong even if not right/precise/careful

? (also drifting ←) individual fib?

8:10 AM go on ch1s ✓

background ~ 300 events (↑ maybe mortality?)

doublet cloud ↑ higher up them in PMA-cell

max max get more affected?

| triplet vs antibody aggregate | how distinguish?

ctrl Perf @ 8:25 → 31 2700 rate rise to 3400

(6min) in practice

Acquisition (continued...)

ctrl NY062 GrmB @ 8:32 am

GrmB + 127

8:37 am → 26 cuts ↑^{dop}.

Shortly after hitting record, there's a fluctuation in eat rate 5 more

6 40

8:54 am → NY062 Perform

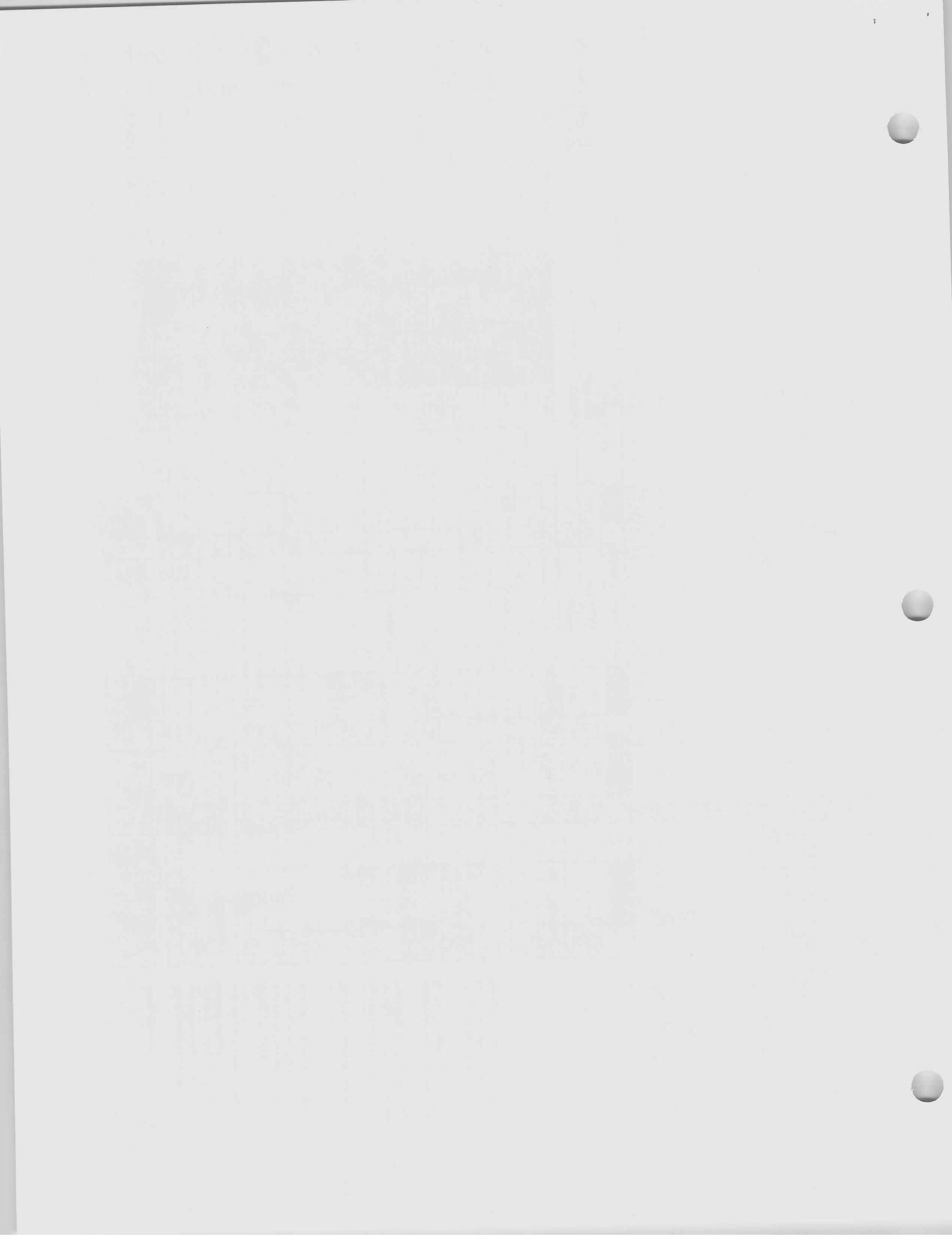
Final sample @ 9:07 am ✓

? the "diminished" sc unstained, start/stop acquisition artefact?

gd T cell SFC panel - Cytokine

#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial/Lot #	During stim!!!	10 15 min (RT)	UD Tetramer 40 min (RT) for 37°C (RT) for 30min	10 Surface staining 40C for 30min	10 then CytoFix/cp	Lysing, then Intra mix RT for 40min intra mix	10
1	UV2			BUV395	CD62L	(DR4E-56)				0.7	7.0	0.25		2.50	
2	UV7			BUV496	CD8	(RPA-78)				1.2	12.0				
3	UV9			BUV562	CD69	(IFN50)				0.7	7.0				
	UV10			BUV615						1.2	12.0				
4	UV11			BUV661	V62	(BS)				0.7	7.0				
5	UV16			BUV737						0.7	7.0				
	V1			BUV805	CD4	(IS32)				1.5	15.0	0.25		2.50	
6	V3			BV421	CD127					1.5	15.0				
	V3			Pacific Blue	CD14	(MS52)				1.5	15.0				
7	V5			Pacific Blue	CD19	(HIB19)				2.0	20.0				
8	V7			BV480	CD161	(RE631)				2.0	20.0				
	V10			BV510	CD45RA	(HIT00)				1.0	10.0				
10	V11			BV605	CD56					0.7	7.0				
	V13			BV650	CCR7					0.7	7.0				
11	V14			BV711	CD122					1	10				
12	V15			BV750	IFNγ	(BZ2)									
	V15			BV786	CCR6	(1149)				1.8	18	1.5		15.00	
13	B2			AlexaFluor 488	hCD14										
14	B3			Spark blue 550	CD3	(SK2)									
15	B4			PE	NKG2D					1.2	12.0	0.25		2.50	
16	B6			PE-CF594	CD26	(MA-261)				0.7	7.0				
17	B8			PE-CY5	CD25	(MA-251)				1.2	12				
18	B10			PerCP-Cy5.5	TNFα	(MAB11)				1.2	12.0	0.25		2.50	
19	B13			PE-vn0770	PDI							2.5		25.00	
20	R2			APC	NGK2A					1.5	15.0				
21	R4			Alexa Fluor 647	hMRL1							(1.0)			
22	R6			APC-F700	CD45ra	(H4A3)									
	R7			Zombie NIR	L(D)										
23	R8			APC-H7e750	CD28										
				APCFire810	CD38	(HIT2)									
Antibody Total										6.8	6.8	Antibody Total	1.6	16	10
And UNSTAINED CONTROLS !!!															
R10 Media										13.7	13.7	Brilliant Stain	50	500	5.25
Pipette draw volume /sample										19.5	19.5	Pipette draw volume /sample	55	65.1	54.25

Notes



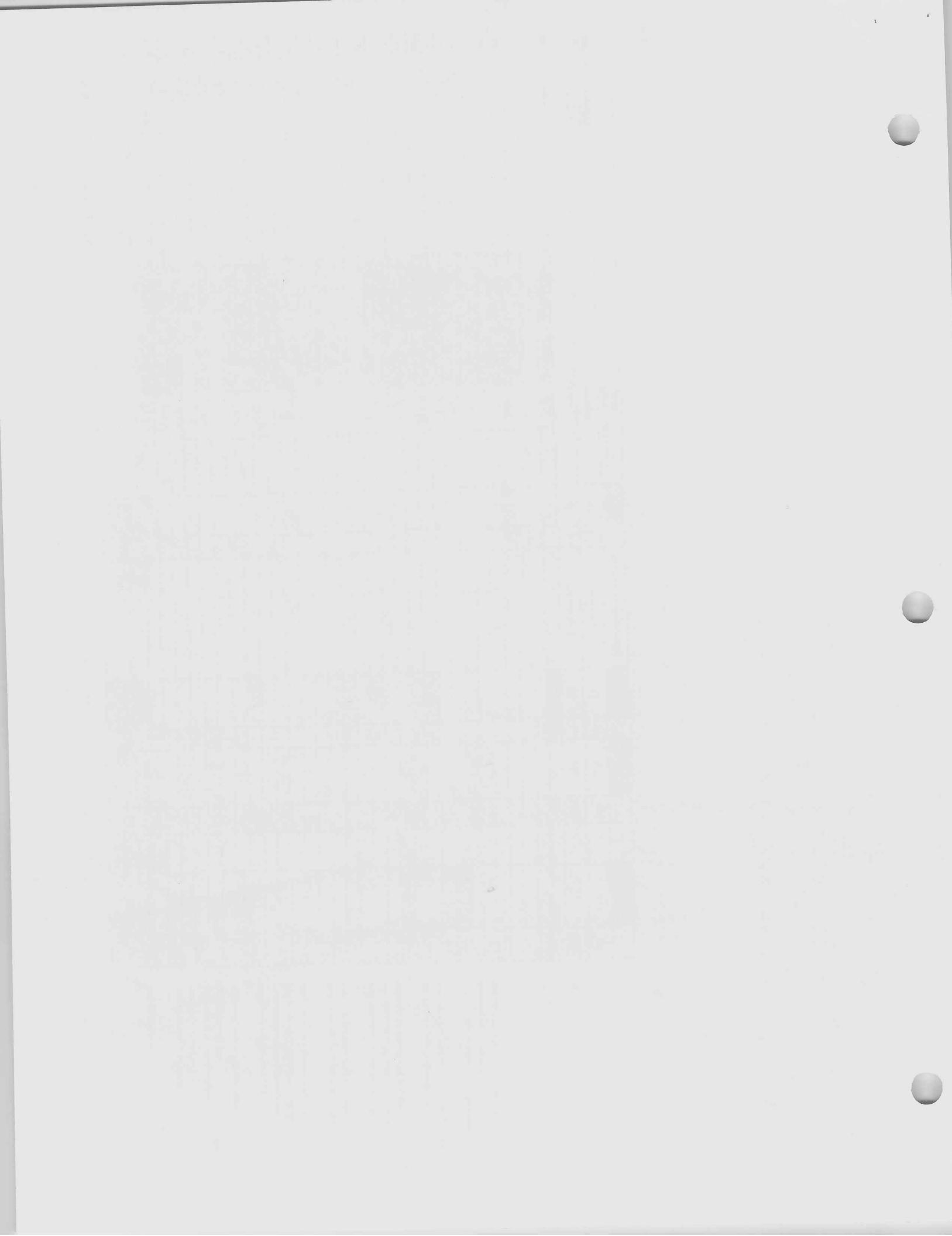
gd T cells - Cytokines

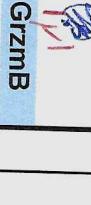
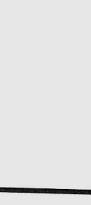
8/17/2022

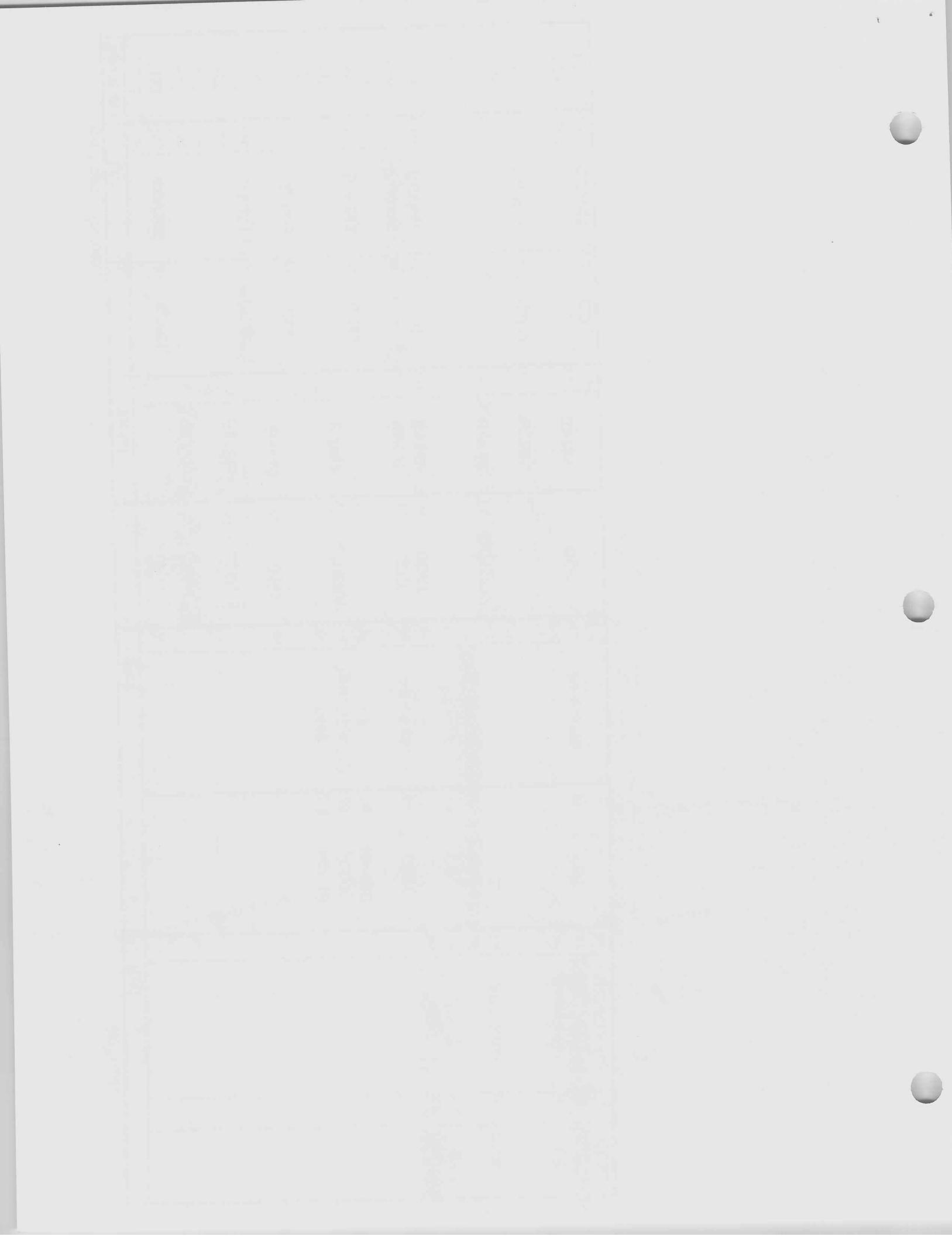
Spectrum	UV		Violet		Blue		Red
373	UV1						
388	UV2	BUV395	[2]	CD62L			
428	UV3			BV421	[4]	CD127?	
443	UV4				V1		
458	UV5				V2		
473	UV6	AF350			V3	PacBlue	
508				CD16?	V4	[1] CD14/19	
514	UV7	BUV496	[2]	CD8	V5	BV480	[3] CD161
525	UV8				V6		
542	UV9				V7		
582	UV10	BUV563	[3]	CD69	B2	AF488	[1-5] hCD1d
598	UV11	BUV615	[3]		B3	SparkBlue 550	[1] CD3
613	UV12	BUV661	[3]	Vd2	B4	PE	[4] NKG2D
664	UV13				B5		
679	UV14				B6	CD56	
697	UV15				B7	CD45RA	
717	UV16				B8	PE-CF594	
738					B9	PerCP-Cy5.5	
750					B10		
760					B11		
783					R1	APC	[3.5] NKG2A?
812					R2	AF647	[3.5] hMR1
		BUV737	[3]	CD27?	R3		
			V14	BV750	R4	CD25	
					R5	TNFα	
						APC-R700	
						CD107a	
						CD38	
		BUV805	[1]	CD4	[3]	PD1	
			V15	BV786	[3]	L/D	
			V16	CCR6	R8	Zombie NIR	[1] CD28?
						APC-Fire 810	[3] CD38



Notes



Spectrum	UV		Violet		Blue		Red	8/17/2022
373	UV1							
388	UV2	BUV395	[2]	CD62L	V1	BV421	[4]	
428	UV3				V2			
443	UV4				V3	PacBlue	[1]	
458	UV5				V4			
473	UV6	AF350		CD16?	V5	BV480	[3]	CD14/19
508								
514	UV7	BUV496	[2]	CD8	V6			
525	UV8				V7	BV510	[1.5]	CD45RA
542	UV9	BUV563	[3]	CD69	V8			
582					V9			
598					V10	BV605	[3]	CD56
613	UV10	BUV615	[3]		V11	BV650	[3.5]	CCR7
664	UV11	BUV661	[3]	Vd2	V12			
679					V13	BV711	[4]	PE-Cy5
697	UV12							
717	UV13					PerCP-eFlour700	[2]	CD25
738								
750	UV14	BUV737	[3]	CD27?	B10			
760					B11			
783						APC	R1	
812	UV15					AF647	R2	
	UV16	BUV805	[1]	CD4	B12		R3	
					B13		R4	
					B14		R5	
						APC-R700		
						Zombie NIR	[3]	CD107A
						APC-Fire 750	[1]	
						APC-Fire 810	[2]	
						CD38	[3]	



August 17th, 2022

$\gamma\delta$ SFC Panels #1

PMA wells: NY062 ctrl

no G2L

CD8 (69) ecty

161 > 45RA > 56

IFNy

CD62L b

α CD8 CBMC
 α CD69 CBMC v1

\sim CD45RA - 1PS

CD86

1. 273 to 274

273
274
275
276
277

7/8/2022

(Dcs)

Monocytes SFC Panel - V2

Spectrum	UV	Violet	Blue	Red
373	UV1			
388	UV2	BUV395	[2] CD18	
428	UV3		V1 BV421	[4] FcεRI
443	UV4		V2	
458	UV5		V3 PacBlue	[1] CD5
473	UV6	A7350	V4	
508	UV7	BUV496	[2] CD19	V5 BV480
514	UV8		V6	[3] CD11a
525	UV9	BUV563	V7 BV510	B1
542	UV10		[1.5]	B2 FITC
582	UV11	BUV661	V8 BV570	B3 SparkBlue 550
598	UV12		V9	B4 PE
613	UV13		V10 BV605	B5 CD16
664	UV14		V11 BV650	B6 PE-CF594
679	UV15		V12	[3.5] CD56
697	UV16	BUV737	V13 BV711	B7 CD11c
717			V14 BV750	B8 PE-Cy5
738			V15 BV785	[4.5] CD86
750			V16	R1 CD123
760				R2 AF647
783				R3
		BUV805		R4 APC-R700
				R5
				R6
				Zombie NIR
				R7 APC-Fire 750
				R8 APC-Fire 810
				[1] L/D
				[2] CD64
				[3] CD38