

June 20th, 2024

SFC Alpha Beta - Infant | Adult PPD Responses

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
Inf956-0 P13m	HO ♀ 4 month			7/1/22		0.3		11 E6	3.3 E6
Inf915-5 UZ8V	HO ♀ 9 month			11/7/22		0.3		5.4 E6	1.6 E6 J&S
CVD-6022	Adult ♂		1SE46	3/9/23		1.0		14 E6	14 E6
Mat-600-C EOTAS	Adult ♀			6/26/19		0.5		2.9 E6	1.4 E6

Thursday

~ 7:15-7:20 am Thaw start
7:37 pm spin down @ 10,200 rpm

7:55 am DNAse start

Biox stain for count

& wait for QC

8:35 done counts

8:58 calcs done 3ml/mL & 2ml/s
9:12 pm Culture Rest In //

3:54 pm count stain #2

5:05 pm spinning down

6:02 pm culture start

(silica glass pan)

8:00pm 8% Gels blocked

8:14 AM: Cells spinning down.
running like on mixer.

8:32 //

9:12 am Zombie

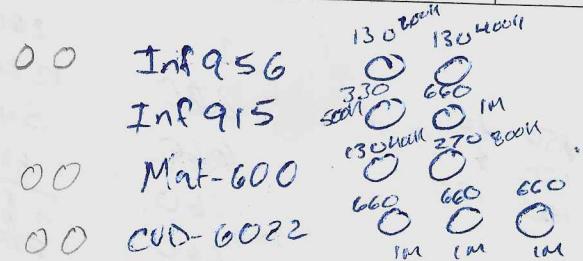
9:39 am Centrifuged Zombie tubes

10:12 Surface Antibody

10:37 Surface wash and 2nd Zombie

10:50 Strep....

11:00 Single colors



15 + 30 + 15 + 40 + 15

11:27 Step off
& Sel's wash

Fix @ 11:47 am
1st ✓ @ 11:57
@ 12:07

12:20 first wash
12:33 end wash

12:55pm Incubation
Intact/Var @ 1
→ 1:35 pm
3rd wash @ 1:38 pm

2:02 pm Done

915 130 μ l for Inf915 Wodd cells

A56 330 μ l 600 μ l 1m 130 μ l unstained
Met 130 μ l 270 μ l 400 μ l
330 μ l 330 μ l 330 μ l
~~670 μ l~~ 670 μ l 670 μ l

330 μ l
33
990
9900
10,890



670

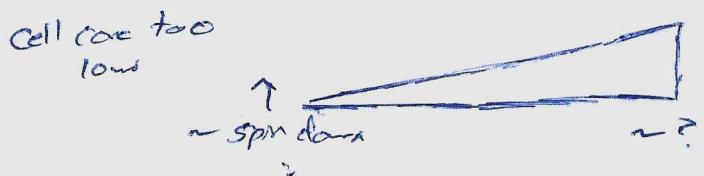
~~450 150 150 150~~
~~150 150 150 150~~
~~2 260 660 260 660~~
~~260 1220 540 600~~
~~660 2680 660 1 ml~~
~~1840 2 ml~~
SETB
8ml →

2 ml
BSL

2 M/mL or something similar for concentration.

Automated highlight →

certain amount 2x cone cells → Spill 2x contingency



Mix for GolgiPlus after wash (does it work?)

/ Normal than # cells & Normal rest conc / Tcone than staining? stim

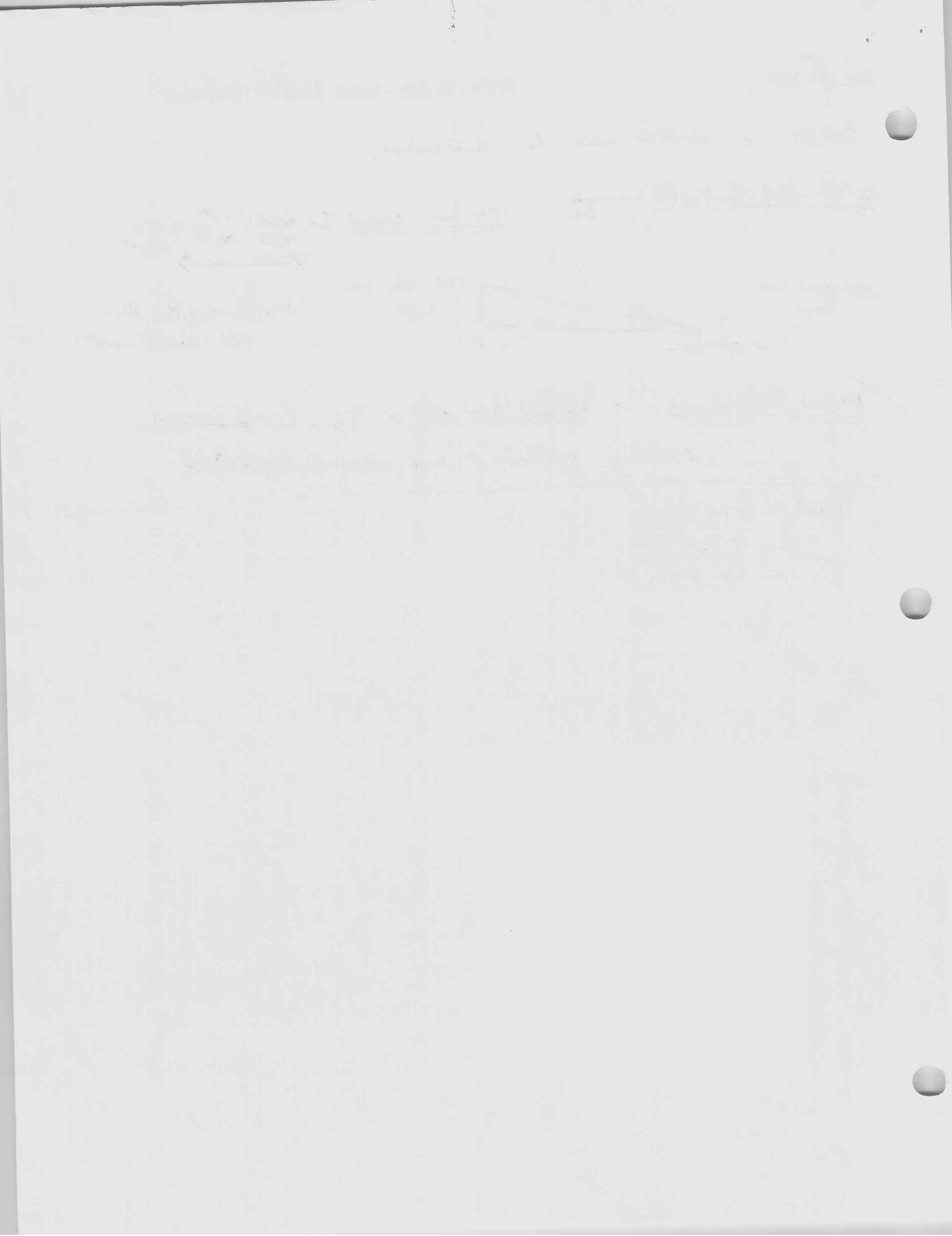
< normally spin down & snap supernatant/media? >

T cell ~ APC ratio

- ~ B cell ratio
- ~ Mon ratio

Lym	B cells	Mon
0.1.1 E7	1.6 E6	1.1 E6
0.5 2.3 E6	9.9 E5	1.1 E6
0.5 5.8 E6	1.7 E6	9.3 E5
Nom 1.6 E6	2.7 E5	6 E5

11	2.7	~24%
2.3	2.1	~91%
5.3	2.6	~44%
1.6	0.87	~54%



FBS

Specimens
4 unstained
28 sec

$$\begin{aligned} * 6 = 54 \quad 6 \text{ ml} &= 54 \quad 1 \text{ ml} = 9 \quad 1.5 \text{ ml} \\ * 2 = 8 \quad 3 \text{ ml} &= 12 \quad \underline{500 \text{ ml}} = 2 \quad 250 \text{ ml} \\ * 2 = 56 \quad 3 \text{ ml} &= \frac{56}{122} \quad \underline{500 \text{ ml}} = 14 \end{aligned}$$

118 12:108 25 ml
 5% PBS Nuclear wash Nuclear fix

~~FBS
wash~~

$$10\% \quad \text{FBS} \quad \rightarrow \quad 5\% \quad 120 \text{ ml}$$

60 ml - 60 ml
w/PBS

$$\begin{aligned} 1.5 \text{ ml} &= 1 \text{ ml} \\ \underline{250 \text{ ml}} &= \underline{500 \text{ ml}} \end{aligned}$$

Nuclear wash

Perm Buffer	12 ml
de H ₂ O	108 ml

Nuclear fix

7 ml of biuret	
22 ml Buffer Perm	

$$\begin{array}{r} 800 \\ \times 10 \\ \hline 8 \text{ ml } 4 \text{ ml Zambac} \end{array}$$

June 21st, 2024

June 21st, 2024									αβ T cell/SCC panel						
#	Filter	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	9	L/D RT 15 min	RT for 30min @4C	ColdStain 1	4°C for 15 min	RBC Lyse, then Fix/Perm	Intranuclear Stain 40 min @RT	1	
1	UV2	BUV395	CD62L	(DRREG-56)				1.0	1.0	1	1				
2	UV7	BUV496	CD8	(RPA-T8)				0.5	0.5		0.5	0.5	0.5		
3	UV9	BUV563	CD69	(FN50)				0.5	0.5		0.5				
4	UV10	BUV615	CCR4	(1G1)				1.5	1.5		0.5	0.3	0.3		
5	UV11	BUV661	V52	(B6)				0.5	0.5						
6	UV14	BUV737	CD4	(SK3)					1.0	1		0.5	0.5		
7	V1	BV421	CD127	(A019D5)					1.0	1					
8	V3	Pacific Blue	CD14	(M5E2)					1.5	1.5					
	V3	Pacific Blue	CD19	(HB19)					1.5	1.5					
9	V5	BV480	CD161	(REA31)					1.5	1.5	<0.8>				
10	V7	BV510	CD45RA	(HI100)				0.5	0.5						
11	V10	BV605	CXCR5	(J252D4)				1.5	1.5						
	V11	BV650							1.0	1					
12	V13	BV711	Va7.2	(3C10)						1.5	1.5				
13	V14	BV750	IFNγ	(B27)					1.5	1.5					
14	V15	BV786	CCR6	(11A9)						1.5	1.5				
15	B2	Alexa Fluor 488	FoxP3							3	3				
16	B3	Spark Blue 550	CD3	(SK7)						1.0	1	0.5	0.5		
17	B9	PerCP-Cy5.5	CD26	(MA261)						2	2				
18	YG1	PE	PD1	(EH12.2H7)						1.2	1.2	0.5	0.5		
19	YG3	PE-Dazzle594	TNFα	(MAB11)						1.2	1.2	1.2	1.2		
20	YG5	PE-Cy5	CD25	(M-A251)						1.2	1.2	0.5	0.5		
	YG8	cFluor-BYG750								0.5	0.5				
21	YG9	PE-vio770	HLA-DR	(L243)						1.0	1				
22	YG10	PE-Fire810	CCR7	(A1)						1.0	1	1.2	1.2		
23	R1	APC	CD39	(A1)											
24	R2	Alexa Fluor 647	IL2	(MO1- IL2)											
25	R4	APC-Cy7	CD107a	(H4A3)				<6>	<1-2500>						
26	R6	Zombie NIR	L/D												
27	R7	APC/Fire 750	CD27	(O323)						1.0	1				
28	R8	APC/Fire 810	CD38	(HTT2)							1.0				
And UNSTAINED CONTROLS !!!				Antibody Total	0.0	Antibody Total	22.9	22.9				11.7	12		
				R10 Media	20.5	Brilliant Stain	45.8	45.8				23.4	23		
				Pipette draw volume	19.5	Pipette draw	65.7	65.7				32.1			

Wash with 1 ml PBS, spin down 1300rpm 8min
800 ul of LiveDead mix (1:2500) @ RT for 15min
Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min

Add CD161 biotin antibody for 10 minutes at RT

Add ColdStain mix, incubate @ 4C for 30min

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

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

Add Streptavidin Mix, incubate @ 4C for 15min

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

1 ml Nuclear FixPerm, incubate @ 4C for 30min
(vortex every 10 minutes)

First Perm/Wash: 2 ml NuclearWash 1500 rpm 6 min

Second Perm Wash: 2 ml NuclearWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min

First Perm/Wash: 2 ml NuclearWash 1500 rpm 6 min

Second Perm Wash: 2 ml NuclearWash 1500 rpm 6 min

Resuspend in 30 ul 0.4% PFA+PS
Cap tubes, wrap rack in foil, store at 4°C

5:56 pm

→ 18 AM

1) Ob conc
2) Total
3) Total
4) Desired
5) Required
6) Increase
7) Max mLs
8) Total mLs per Tube
9) SpinDown Tubes

Specimen	Date	Cells	Volume	Concentration	Volume mLs	Volume mLs	Max mLs per Tube	SpinDown Tubes
ND050	14-Jun-2024	6.5e+06	1.0	3e+06	2.2	1.2	2	FALSE
CVD6022	20-Jun-2024	1.4e+07	1.0	3e+06	4.7	3.7	2	2.3
INF915	20-Jun-2024	1.6e+06	0.3	3e+06	0.53	0.23	2	FALSE
INF956	20-Jun-2024	3.3e+06	0.3	3e+06	1.1	0.8	2	0.55
MAT600	20-Jun-2024	1.4e+06	0.5	3e+06	0.47	-0.033	2	TRUE
								0.23

5.23
1.57
6.80

8

$$\begin{aligned}130 &= 400 \text{ K} \\270 &= 800 \text{ M} \\330 &= 10 \text{ L}\end{aligned}$$

1.3

~~2.7~~

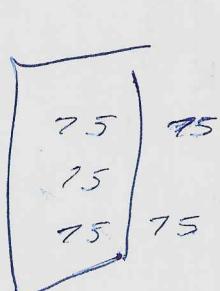
3.3

$$600 = 2 \text{ M}$$

$$\begin{aligned}6.7 \mu\text{l} &= 10 \mu\text{l} \text{ in } 1 \text{ ml} \\5 \mu\text{l} &= 5 \mu\text{l} \text{ in } 1 \text{ ml}\end{aligned}$$

2 ml
1 ml

$$(0.7 = 75 \mu\text{l})$$



$$\begin{array}{ccc}1145 & 1150 & 1350 \\ \times 2 & \times 2 & \times 3 \\ 2290 & 2300 & 2700 \\ \times 10 & \times 10 & \times 5 \mu\text{l} \\ 230 \mu\text{l} & 23 \mu\text{l} & 13.5 \mu\text{l} \end{array}$$

June 21st, 2024

CVDR22 → ch1
CVDR22 → ch1
CVDR22 → ch1
CVDR22 → ch1

#	Filter	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	9	1/1D RT 15 min	RT for 10 min	ColdStain 30min @4C	9	4°C for 15 min	RBC Lyse, then FixPerm	Intranuclear Stain 40 min @RT	9	αβ T cell SFC panel
1	UV2	BUV395	CD62L	(DRG-56)					1.0	9						
2	UV7	BUV496	CD8	(RPA-T8)					0.5	4.5						
3	UV9	BUV563	CD69	(FN50)					0.5	4.5			0.5	4.5		
4	UV10	BUV61S	CCR4	(1G1)					1.5	13.5			0.5	4.5		
5	UV11	BUV661	V52	(B6)					1.5							
6	UV14	BUV737							0.5	4.5			0.3	2.7		
7	UV16	BUV805	CD4	(SK3)												
8	V1	BV421	CD127	(A019D5)					1.0	9			0.5	4.5		
	V3	Pacific Blue	CD14	(M5E2)					1.0	9						
	V3	Pacific Blue	CD19	(HIB39)					1.5	13.5						
9	V5	BV480	CD161	(RA631)					1.5	13.5			Strep			
10	V7	BV510	CD45RA	(H1100)					0.5	4.5			<0.8>			
11	V10	BV605	CXCR5	(I252D4)					1.5	13.5						
12	V11	BV650														
13	V13	BV711	Va7.2	(3C10)					1.0	9						
14	V14	BV750	IFNV	(B27)									1.5	14		
15	V15	BV786	CCR6	(11A9)					1.5	13.5						
	B2	Alexa Fluor 488	FoxP3													
16	B3	Spark Blue 550	CD3	(SK7)									3	27		
17	B9	PercP-Cy5.5	CD26	(M-A261)					1.0	9			0.5	4.5		
18	YG1	PE	PD1	(EH12.2H7)									2	18		
19	YG3	PE-Dazzle594	TNFa	(MAB11)					1.2	10.8			0.5	4.5		
20	YG5	PE-Cy5	CD25	(M-A251)									1.2	11		
	YG8	cFluor-BV750							1.2	10.8			0.5	4.5		
21	YG9	PE-W770	HLA-DR	(L243)					0.5	4.5						
22	YG10	PE-Fire810	CCR7						1.0	9						
23	R1	APC	CD39	(A1)					1.0	9						
24	R2	Alexa Fluor 647	IL2	(MO1- R4)												
25	R4	APC-C770	CD107a	(H4A3)					<6>	#####			1.2	11		
26	R6	Zombie NIR	L/D													
27	R7	APC/C770	CD27	(O323)												
28	R8	APC/Fire 810	CD38	(H1T2)												
And UNSTAINED CONTROLS !!!							Antibody Total	0.0	Antibody Total	22.9	206.1			11.7	105	
							R10 Media	20.5	Brilliant Stain	45.8	412			23.4	211	
							Pipette draw volume	19.5	Pipette draw	65.7				32.1		

1000 μl 400 μl

$$\begin{array}{r} 800 \\ \times 10 \\ \hline 8000 \end{array}$$

8m 4y1 ~~zombie~~

Infant PBMC AB T cells

6/21/2024

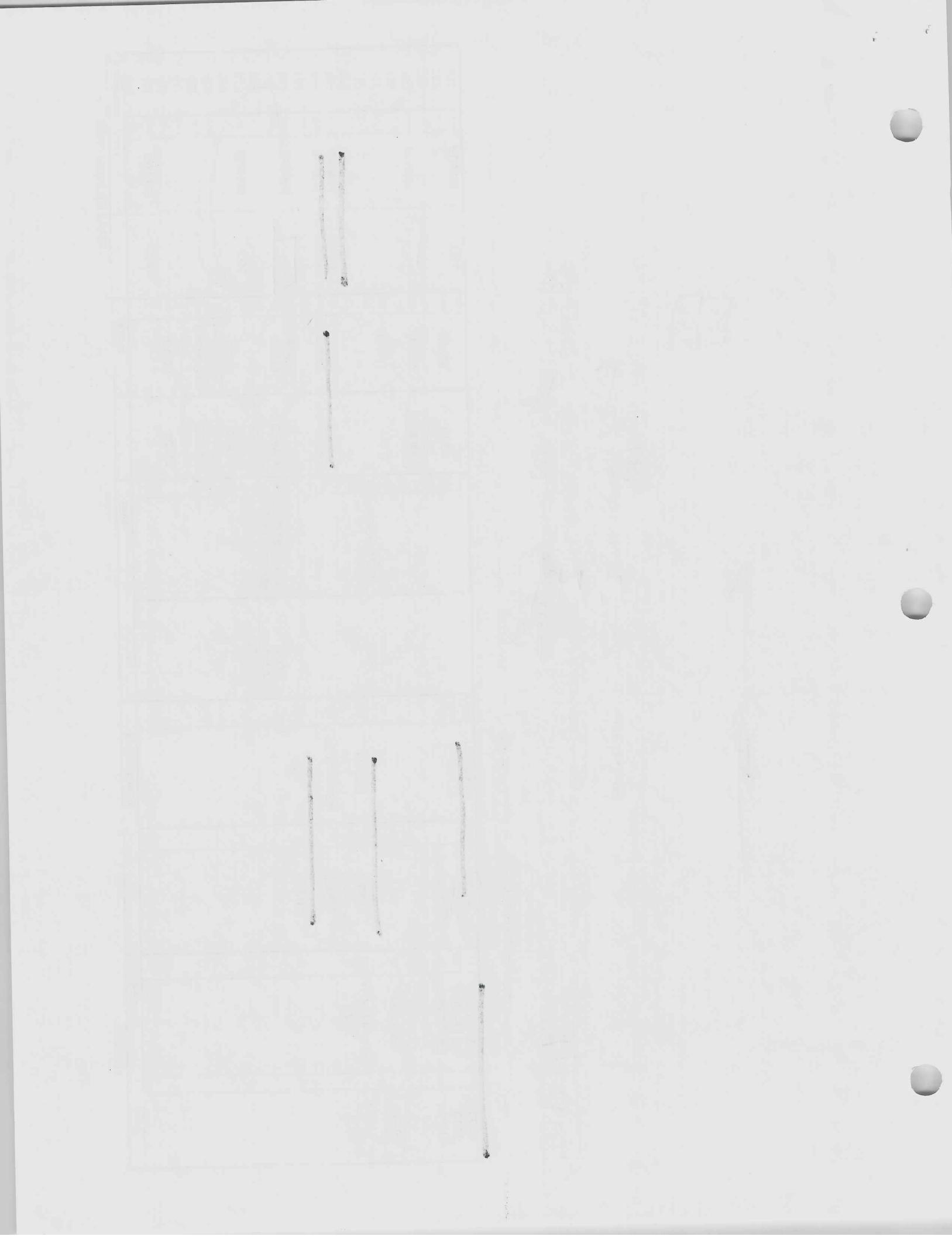
Spectrum	UV		Violet	Blue	Yellow-Green	Red
373	UV1					
388	UV2	BUV395	CD62L			
428	UV3					
443	UV4		BV421	CD127		
458	UV5				CD14/19	
473	UV6					
508						
514	UV7	BUV496	CD8			
525	UV8					
540						
582	UV9	BUV563	CD69			
598						
613	UV10	BUV615	BV510	Alexa Fluor 488		
664	UV11	BUV661	BV570	SparkBlue 550		
679					FoxP3	
697	UV12		BV605	CD3		
717	UV13		CXCR5			
738			BV650			
750	UV14	BUV737	Va7.2.			
760			BV711			
783	UV15		IFNγ			
812	UV16	BUV805	CD4			
					PE	
					PE-Dazzle594	
					PE-Cy5	
					CD26	
					PD1	
					TNFα	
					CD25	
					CD39	
					IL-2	
					CD107a	
					APC	
					Alexa Fluor 647	
					R1	
					R2	
					R3	
					R4	
					R5	
					R6	
					R7	
					R8	
					Zombie NIR	
					APC-Fire 750	
					APC-Fire 810	
					Viability	
					CD27	
					CD38	

Ad (2) (2)
 29 colors
 5 cytoines + 27 + 1 unstained
ct₁ 22 SES + 27 + 1 unstained
 (8) (2)

[15]
 [35]

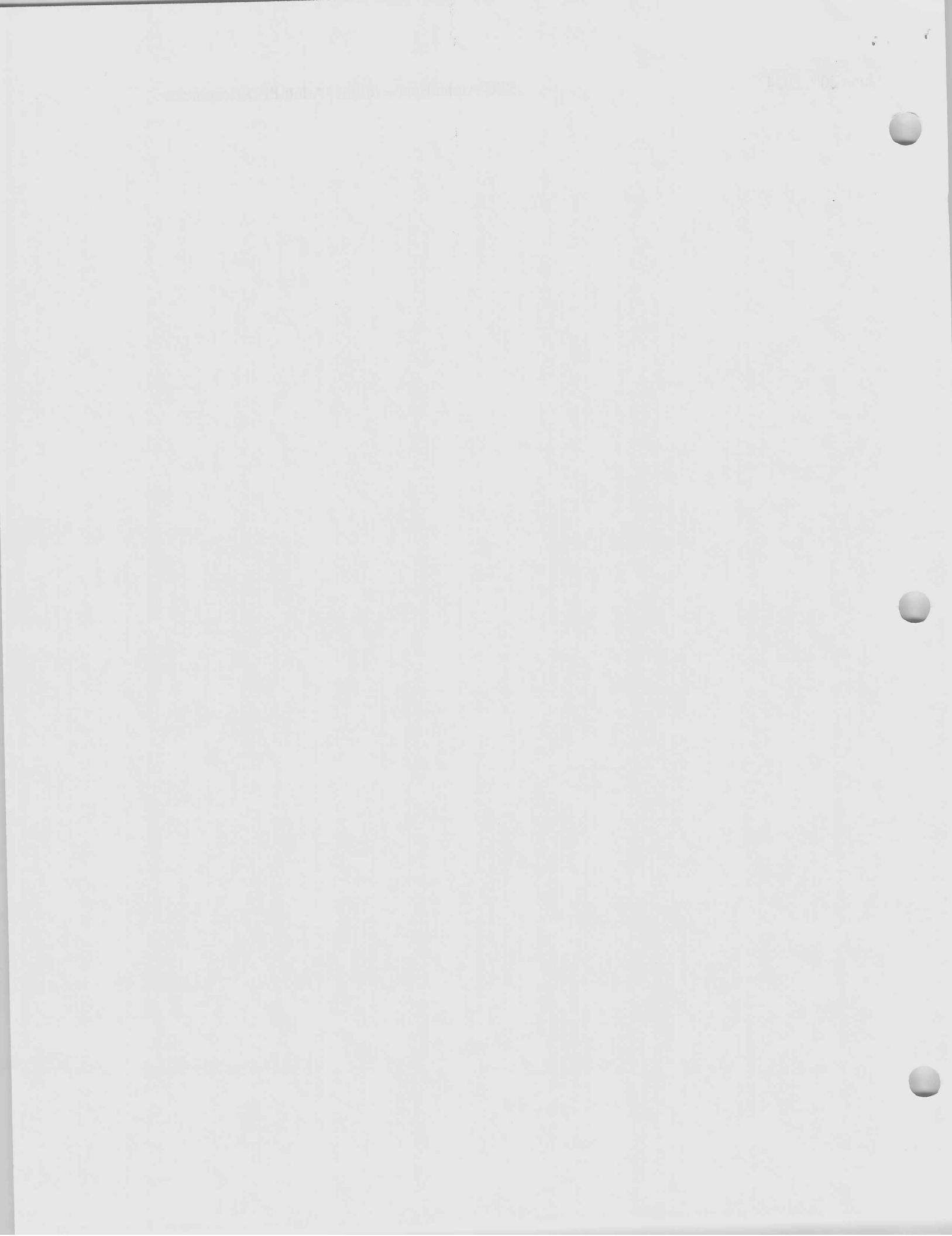
PPD Test + PE-Fire810

4/1 CD107a



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