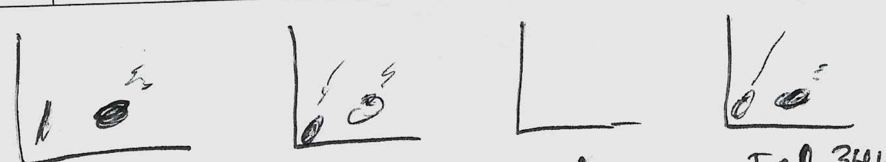
Longitudinal Alpha Beta

ber 5th, 2023

	men	Status	Location	Conc	Date	Notes	Volume	Lym 1:100	Lym+Mon	Total
	Inf305-8 a-3	Cord HU ♀ SM2W (1)	Box 9A, E5	<11.17>			2	8.85	11.4	~~9.4~~ 9.4 (22.8 / 20.8)
200c.	Inf 160-7 a-1	4 months HU ♀ S85E (2)	Box 2A, D5	<15.67>		Pale	0.5	6.62	8.16	4.08 −.50 3.58
600c.	Inf 076-3 a-1	9 months HU ~~HE~~ ♀ KWYB (3)	Box 4A, B7	<26.37>		Bubbly?	0.5	4.09	5.29	2.65 −.50 2.15
800c. 30µL	Inf352-0 a-1	Cord HEU-lo ♀ STW2 (3)	Box 11A, E3	<11.37>			2	6.59	9.94	9.88 ~~[illegible]~~ 9.94
430c.	Inf 247-3 a1	4 months HEU-lo ♀ ~~ST60~~ (?)	Box 4A, D3 SGFB	<15.01>		Monocytes colored	0.5	6.95	8.26	4.13 −.50 3.63
450c.	Inf344-1 a-2	9 months HEU-lo ♀ ST60 (4)	Box5A, E8	<25.37>			0.5	4.28	4.99	2.50 −.50 2.00
490c.	NY068	Adult scs (1)		1.5E7	8/15/23		2	6.39	8.46	16.92 → ~~[illegible]~~ 6.46

start @ 8:53 pm 9:05 spin
9:23 DNAse for cord 1
9:53 stain for count
10:04 wall → 10:12
10:18 Guava count start
10:31 done



11:07 Incubation Start

0804 collecting
3:23 cold 8:47 cold
8:52 done

9:11 spin @ 1200 rpm, 10 min
9:57 am → 10:04 AM
10:04 wall count →
10:18 am clean

11:57 resume post CrisTaln & Dilution Calc sheet

12:22 error discovered respin

1pm Incubation Start no CD107a

10 swirls, collect, add 1 ml hot R10
60 sec tap
add 2 ml hot R10
7 swirls, collect, add 1 ml cold r10
place ice for 15-20 min
Add 2 ml cold R10
10 swirls, collect.

Lym bit right shifted →

	Overnight → count II		Volume	Ly	LyM	
700c.	Inf 305	<9.29>	2	8.94	10.4	17.88
330c.	Inf 160	<20.8>	0.5	4.86	5.51	2.43
450c.	Inf 076	<31.97>	0.5	3.08	3.43	1.54 *
750c.	Inf 352	<10.36>	2	5.53	7.55	11.06
480c.	Inf 247	<19.44>	0.5	5.15	5.22	2.57
320c.	Inf 344	<30.25>	0.5	3.26	3.45	1.63
514c.	NY068	<20.20>	2	4.94	6.29	9.88

Bref/Nonensm
@ 3:20 pm samples
@ 3:26 pm scs

added " → 3:46 pm 2nd half "

1 → 1 am (24 10) <12 hr>
3 am (2+12) 14 hr
4 am (2+13) 15 hr

5 µl - 500 µl
10 µl - 1000 µL 1 mg/mL

SEB 2.5 - 500 µL
5 µg/mL - 1 mL

$(10)(x) = (0.4)$ VL · C

10 (x) 0.4 (10)

9
×5
45

46
×4.8
28.8

10
×5
50

1
×2.5
2.5

45
31.3
126.3

1:1:19
×7
7:7:133

Arrived @ 01:51 am
Coffee @ 1:57 am
2:47 Ab start
3:44 reagents prepped

Dropped 2 tubes, lost 1/3 → 2/3rd volume
both were cord fortunately.

~~1:00 → PPD 0.5 → Media~~
< 4:05 am spin down >
4:22 L/D → 4:37 am
Spin @ 4:44 am

Strept start @ 5:05 → 5:15
5:06 am Inf352 ctrl L/D →
// 5:10 sc's spin //
5:22 pm L/D spin ← 5:20 pm Hot → 5:50 pm
Spin @ 5:57 am
> Hot & cold @ 5:32 am → 6:02 am Spin @ 6:05 am
5:41 am Hot sc's → 6:11 am Spin @ 6:18 am
5:51 am Cold Sc's → 6:21 am Spin @ 6:26 am
6:16 AM cold sc start → 6:46 am //

RBC lyse @ 6:51 am
Spin @ 6:58 am

NY068 ctrl → CCR4, CXCR5 & Ki7.2 200 µL
NY068 SEB → CD69, TNFα, IFNg, IL-2 200 µL
Inf305 ctrl → All others 150 µL

7:12 strep added
7:23 Bead stain cold 4°C (new bottle)
→ 7:53 //
> Spin @ 7:30 AM //

FixPerm @ 7:45 → 55 → 8:05 → 8:15 am
→ methanol colder tank
Beads spin @ 7:53 [1 ml FBS wash]
Bead FixPerm @ 8:00 → 8:10 → 8:20 → 8:30?
8:05 → Merged w/ rest samples for vortex's

8:24 AM: First Wash [Samples & cell sc's]

2nd wash @ ~~8:47 AM~~ 8:50 AM
resuspending sc's in ~~20 µL~~ 15 µL

Intrac @ 9:10 am → 9:50 am
unst/sc's stained @ 9:26 w/ 15/25 µL

3rd wash @ 9:48 am
Final wash @ 10 am

Beads 30 mins? 2 µL for FoxP3
500g 5 min

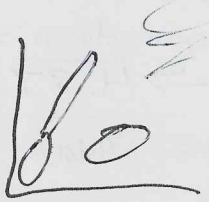
Template setup @ 1:04pm

R3 failed x2 days row ← clump cells in line??

1:08pm event cal 430 c 27 µl/min ←
nope, R3 > % threshold change

← running control for 5 minutes >
H2O @ 1:21 pm

4:30 pm 76 µL/min on High
↑50 µL 30 sec



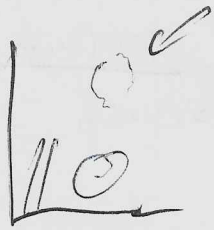
¿CCR4 low?

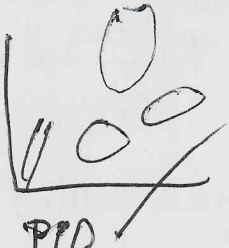
CD161 ← Brad crap ← ← slice & edit

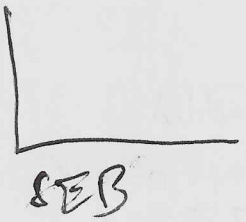
CXCR5 ✓ visible
PD1 ← card activated next time...
HLA-DR Pop in Lym

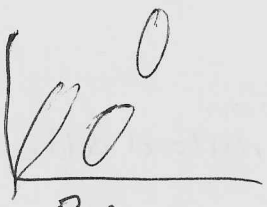
Monocyte cloud moved upwards.

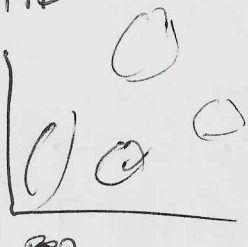
Samples SOS

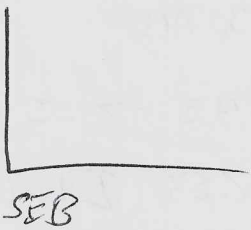

305 ctrl


PPD


SEB


352


PPD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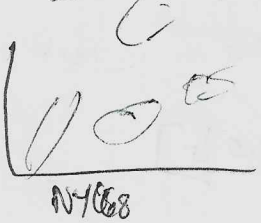
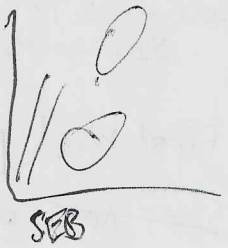

NY168


SEB

Und 2 sc @ 5:21 pm

2.43/2 = 5

	Volume	Conc	Total	Add R10 to get to 2M/mL			uL/uL
INF305	2	8.94	17.88 ← all 2	2.47	6.94	**PPD**	1
INF160	0.5	4.86	2.43 ← Media, PPD	1.93	0.715	**SEB**	1
INF076	0.5	3.08	1.54 ← just PPD	1.04	0.27		
INF352	2	5.53	11.06 ← all 2	0.765	3.53		
INF247	0.5	5.15	2.575 ← Media, PPD	2.075	0.785		
INF344	0.5	3.26	1.63 ← just PPD	1.13	0.315		
NY068	2	4.94	9.88 ← all 1.5	0.47	2.94		

1.5 or round up?

Tube Cells	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1**	**1.2**	**1.4**	**1.6**	**1.8**	**2**
INF305	0.2	0.25	0.3	0.35	0.4	0.45	0.5	0.6	0.7	0.8	0.9	1
INF160	0.2	0.25	0.3	0.35	0.4	0.45	0.5	0.6	0.7	0.8	0.9	1
INF076	0.2	0.25	0.3	0.35	0.4	0.45	0.5	0.6	0.7	0.8	0.9	1
INF352	0.2	0.25	0.3	0.35	0.4	0.45	0.5	0.6	0.7	0.8	0.9	1
INF247	0.2	0.25	0.3	0.35	0.4	0.45	0.5	0.6	0.7	0.8	0.9	1
INF344	0.2	0.25	0.3	0.35	0.4	0.45	0.5	0.6	0.7	0.8	0.9	1
NY068	0.2	0.25	0.3	0.35	0.4	0.45	0.5	0.6	0.7	0.8	0.9	1

Parallel

Tube Volume:	**0.2**	**0.25**	**0.3**	**0.35**	**0.4**	**0.45**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**	**1**
PPD	2	2.5	3	3.5	4	4.5	5	6	7	8	9	10
SEB	1	1.25	1.5	1.75	2	2.25	2.5	3	3.5	4	4.5	5
GolgiPlug	1	1.25	1.5	1.75	2	2.25	2.5	3	3.5	4	4.5	5

→ CD107a

3 — 4.8 — 6

Did we add CD14?

September 7th, 2023 αβ T cell SFC panel - Longitudinal

#	Filter	Single color (ul)	Ref ctrl type	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	15	L/D RT 15 min	RT for 10 min	HotStain 30min @37C	15	ColdStain 30min @4C	15	4°C for 15 min	RBC Lyse, then Fix/Perm	Intracellular Stain 40 min @RT	15
1	UV2				BUV395	CD62L	(DREG-56)								1.2	18				
					AF	AF-UV6														
2	UV7				BUV496	CD8	(RPA-T8)								0.7	10.5				
3	UV9				BUV563	CD69	(FN50)								0.5	7.5				
4	UV10				BUV615	CCR4	(1G1)						2.5	37.5					1	15
5	UV11				BUV661	Vδ2	(B6)								0.7	10.5			0.1	
6	U14				BUV737	CXCR3	(1C6/CXCR3)								<1.3>	#####				
7	UV16				BUV805	CD4	(SK3)						1.5	22.5					0.5	7.5
8	V1				BV421	CD127	(A019D5)						1.5	22.5						
9	V3				Pacific Blue	CD14	(M5E2)								2.0	30				
	V3				Pacific Blue	CD19	(HIB19)								2.0	30				
10	V5				BV480	CD161 -Biotin	(REA631)					1.5					Strep:	0.8		
11	V7				BV510	CD45RA	(Hi100)								0.7	10.5				
					BV570															
12	V10				BV605	CXCR5	(J252D4)						2.5	37.5						
13	V11				BV650	CCR7	(G043H7)						1	15		0			0.7	10.5
14	V13				BV711	Va7.2	(3C10)								1.2	18				
15	V14				BV750	IFNγ	(B27)												1.5	22.5
16	V15				BV786	CCR6	(11A9)						1.5	22.5						
17	B2				Alexa Fluor 488	FoxP3	(236A/E7)												5	75
18	B3				Spark blue 550	CD3	(SK7)								1.2	18			0.1	1.5
19	YG1				PE	PD-1	(EH12.2H7								1.2	18			0.5	7.5
20	YG3				PE-Dazzle594	TNFα	(MAB11)						<1.2>						1.2	18
21	YG5				PE-Cy5	CD25	(M-A251)						1.2	18					0.5	7.5
22	B9				PerCP-Cy5.5	CD26	(M-A261)												2.5	37.5
23	YG9				PE-vio770	HLA-DR	(L243)								0.8	12				
24	R1				APC	CD39	(A1)						1.2	18						
25	R2				Alexa Fluor 647	IL2	(MQ1-												1.2	18
26	R4				APC-R700	CD107a	(H4A3)		<6>	######										
27	R6				Zombie NIR	L/D					<1:2500>									
28	R7				APC/Fire 750	CD27	(O323)						2	30						
29	R8				APC/Fire 810	CD38	(HIT2)						1.6	24						
							Antibody Total		0.0		Antibody Total		16.5	247.5	12.2	183.0			14.8	222
And UNSTAINED CONTROLS !!!							R10 Media		20.5		Brilliant Stain		50	750	50	750			5.7	85.5
							Pipette draw volume /sample		19.5		Pipette draw volume /sample		63.5 (61.5)		59.2				20.5	

Zombie NIR	17		
0.8	13.6	15	PBS
	5.44	6	Zombie

Streptavidin	16
PBS	295.5

	Total	FBS	RBC Lyse	Fix	Perm	PFA
Number Samples	15	120	4.5	15	120	0.45
Number Unstained	9	36	2.7	9	36	0.27
Number Surface SCs (29)	22	22	0	22	88	0.44
Number Intracellular SCs	6	6	0	6	48	0.12
Total		184	7.2	52	292	1.28
		165.6			262.8	

FBS	92
PBS	92

PFA	0.128
PBS	1.152

Perm	29.2
Water	263

RBC	0.72
Water	6.48

Fix	13
Diluent	39

2:39 sc tubes

Bring in Vac Filters

Simplified Protocol

Thaw cells, DNAse Cord, count.
Resuspend at 1-2E6/ml of R10, rest cells for 9 hrs in a 6/12 well plate
Collect, count, aliquot cells 1.5E+6 Cells/ 5ml polystyrene tube
Add antigens, stim antibodies, and DuringStim antibodies in 500 ul R10
Cap and incubate at 37°C for 2 hours
Add golgi block mix, and continue to incubate for <10-13> hours

Wash with 2 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 to it HotStain mix, incubate @37C for 30 min
Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

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

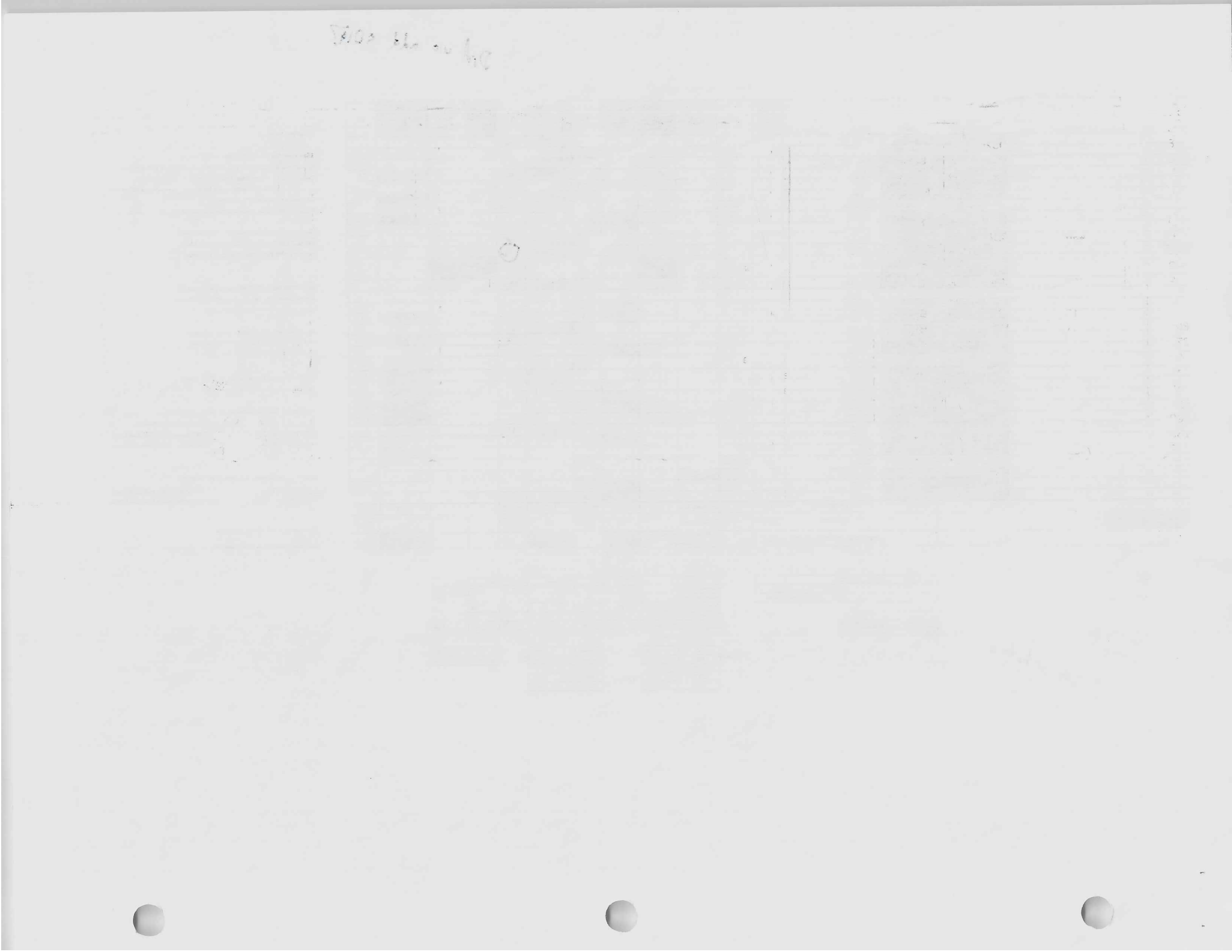
Add Streptavidin Mix, incubate @ 4C for 15min
Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

1 ml Nuclear FixPerm, incubate @ 4C for 30min
(vortex every 10 minutes)
First PermWash: 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 PermWash: 2 ml NuclearWash 1500 rpm 6 min
Second Perm Wash: 2 ml NuclearWash 1500 rpm 6 min

Resuspend in 100 ul 0.4% PFA-PBS
Cap tubes, wrap rack in foil, store at 4°C

Unst



AB T cells

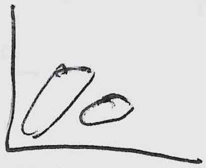
September 6th, 2023

Spectrum	UV				Violet				Blue				Yellow-Green				Red			
373	UV1																			
388	UV2	BUV395	[50]	CD62L																
428	UV3				V1	BV421	[45]	CD127												
443	UV4				V2															
458	UV5				V3	PacBlue	[55]	CD14/19												
473	UV6	AF			V4															
508					V5	BV480	[55]	CD161	B1											
514	UV7	BUV496	[55]	CD8																
525					V6				B2	AF488	[55]	FoxP3								
542	UV8				V7	BV510	[55]	CD45RA	B3	SparkBlue 550	[50]	CD3								
582	UV9	BUV563	[55]	CD69	V8	BV570			B4				YG1	PE	[50]	PD1				
598					V9				B5											
613	UV10	BUV615	[45]	CCR4	V10	BV605	[50]	CXCR5	B6				YG3	PE-Dazzle594	[60]	TNFα				
664	UV11	BUV661	[50]	Vδ2	V11	BV650	[50]	CCR7	B7								R1	APC	[55]	CD39
679									B8				YG5	PE-Cy5	[40]	CD25	R2	AF647	[52]	IL-2
697	UV12				V12				B9	PerCP-Cy5.5	[45]	CD26					R3			
717	UV13				V13	BV711	[55]	Va7.2	B10								R4	APC-R700	[55]	CD107a
738									B11								R5			
750	UV14	BUV737	[40]		V14	BV750	[60]	IFNγ												
760									B12								R6	Zombie NIR	[55]	Viability
783	UV15				V15	BV786	[50]	CCR6	B13				YG9	Pe-Vio770	[50]	HLA-DR	R7	APC-Fire 750	[45]	CD27
812	UV16	BUV805	[50]	CD4	V16				B14								R8	APC-Fire 810	[50]	CD38

Acq samples @ 5:21 p.m. med speed ↑ 70 μl volume set

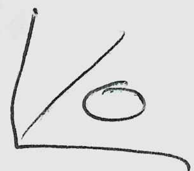
13688 event rate / 200 abort μL Mon 2 doublet visible.
38.43 / min [66 μL]

<305 PPD 1/4 loss volume>

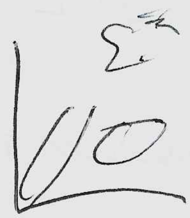


Inf 160 pobrecito

PPD > median aliveness



Inf 76



352 ←

mon increase in size.

? interesting

247

Done @ 6:05

9:59 am Unmix → 10.98 complete, CD27 hit

somedegr TNFa Spreading error zombie

247 → Mon ← PPD wells enriched / retained

CD27 * CD3 ≠ SEB wells as a demarker

CD3 * CD161 ← delineate NKs going to need a lot more intrac CD3 for SEB

352 card is off ≠ CD3% ← contaminating CD127

DNs ← partially the CD3+ NKs // 45RA + 38

Aha!

CD3

CD27

→ CD3

CD161

T1/T17's → No IFNg or TNFa
change CD69, no IL-2

bulk signal is NKs

No PPD activity in DNs.

1.57% PPD CD69+ TNFa+ NY068 1.57

TNFa fuzzies @1.2

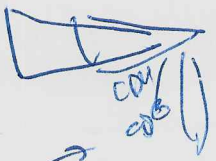
Melting CD69 also
4 months
NY 68
Inf 160
~24

NY068 remarkable issue w/ TNFa
Vs BUS10 (need to ↓ amount?)

27 degraded on 305
Vaya?

HB 4 months
Not great
Inf 160 → TNFa prod CD4
0.60 0.62
305 → 0.016 ↑ 0.055
NY068 → 0.01 ↑ 1.57

Cord CD8s ≠ responsive to SEB ← tiny bit by DN's T17s.



cord CD4's make TNFa, not IFNg to SEB
& IL-2

Strathmann polymme slippage?