

September 23<sup>rd</sup>, 2024

Specimen	Status	Location	Conc	Date	Notes	Volume	CD45+	Total	Resuspension
NY051	Adult		$4 \times 10^7$	6-6-2014	No clumping, pellet looked less 40m	3	8.6 E6	26.0 E6	↑ 8.3 mL

Monday

Thaw @ 10:27 AM

DNAse @ 10:44 AM w/ 50µ

Stain Added @ 10:51 AM

@ 11 AM both 5 & 4L occupied, used 3L

@ 11:17 AM into rest access 8 tubes.

→ up to 13 tubes @ 5:17 pm

5:21 pm SEB & CD107 added

120 pl 1:1:19

1:33

19:14 pm Gelgr Added

Tuesday

6:33 AM ab spin 6:40 AM Abs start

7:13 AM Abs + FBSt + Zentac Made

7:18 AM cells out

7:25 am spin samples

7:39 am h/d

7:47 am SC spin

7:51 h/d spin

8:18 am 1st Samples (all + p added) spin @ 8:51 am

8:25 am 1st sel's spin ✓

8:42 am sel's cold 9:14 am spin ✓

9:02 am Cold samples

+ RBC lysis @ 9:33 am

9:36 am spin ✓

9:31 am → 41 → 51 → 10:01

9:46 am start → 10:01 am

10:10 am Samples Fix → 20 → 30 → 40

10:12 am sel's 1st perm (resting in it since 10:01)

10:27 am 2nd wash Perm sel's

10:50 am Samples 2<sup>nd</sup> wash

10:52 sel's intra

10:59 am Full intra

Final wash @ 11:51 AM

Done @ 12:01 PM

Arg @ 6:16 pm

Squashed monocytes ←  
(considering 10 years old...)

SEB works ✓

Minimal vol2 present (ditto Va 7.2)

(I don't see BV420 !!) ← Need to Fix!

< BV650 @ 1 pl seems not separated >

Icos staining as a creep →

< Low IFNg prod overall > but bright ~

CD8 stain good / strong "CCR3 signal force" ✓  
signal on CD25 ✓

CD107a titration is nice!

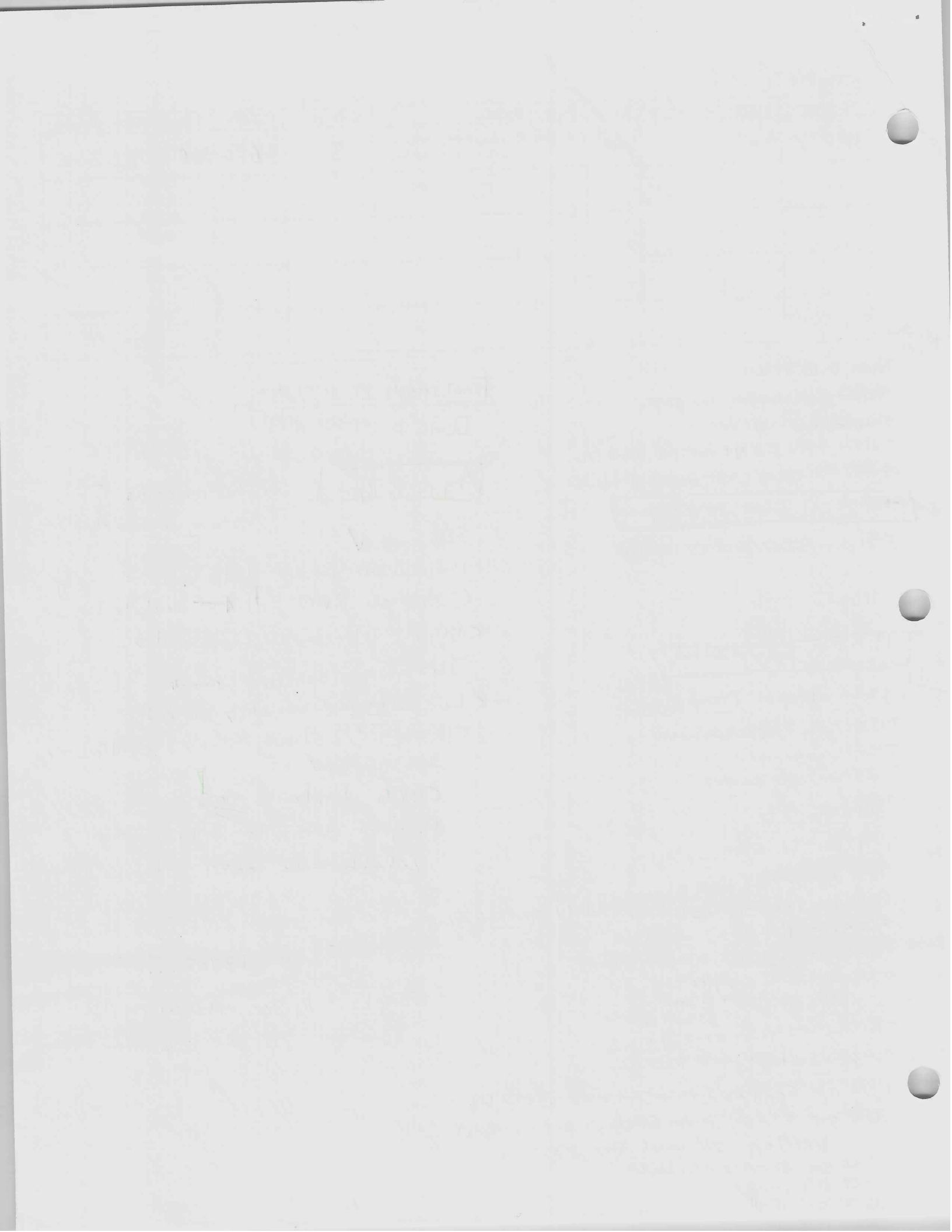
Plenty of dead cells in lymph paper

✓ Patchy nice CCR7 stain on Frest10

No real signal for CD123 pre  
or for RB705...

Samples @ 10:300 cells sec on 11.5h

Done @ 7:42 pm acquisition



September 23rd, 2024

#	Filter	Fluorochrome	Marker	Clone	Dose	During stim!!!	6	L/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	6	Cold Stain 30min @4C	6	4°C for 15 min	RBC lysis, then FixPerm	Intranuclear Stain 40 min @RT	6
1	UV7	BUV395	CD62L	(DREG-56)	1												
2	UV9	BUV563	CD69	(FNU50)	2							1.2	7.2				
3	UV10	BUV615	CD4	(1G1)	2					1.8	10.8	0.5	3			0.8	4.8
4	UV11	BUV661	CD38	(B6)	2							0.5	3			0.1	0.6
5	UV14	BUV737	CD4	(SK3)	1					1.5	9	1.2	7.2			0.7	4.2
6	UV16	BUV805	CD14	(M5E2)	2							1.5	9				
7	V1	BUV421	CD19	(H1B19)	2							1.5	9				
8	V3	Pacific Blue	CD161	(REAG31)	1					1.5	9	0.5	3				
9	V5	BUV480	CD161	(REAG31)	1												
10	V7	BUV510	CD45RA	(H1100)	1												
11	V10	BUV605	CD45RA	(H1100)	1												
12	V11	BUV650	CD127	(J252D4)	2					2.5	15	1.0	7.2			1.5	9
13	V13	BUV711	ICOS	(B27)	1							0.5	3			3	18
14	V14	BUV750	IFN $\gamma$	(B27)	1							0.5	3			0.5	3
15	V15	BUV786	CCR6	(1A9)	1					1.2	7.2					1.5	9
16	B2	Alexa Fluor 488	CD3	(SK7)	1												
17	B3	Spark Blue 550	CD3	(SK7)	1												
18	B4	Spark Blue 574	CD26	(M-A261)	1							1.2	7.2			3	18
19	B9	PerCP-Cy5.5	CD26	(M-A261)	1							0.5	3			0.5	3
20	VG1	PE	PD1	(EH12.2H7)	1							1.0	6			1.5	9
21	VG3	PE-Dazzle 594	TNFR	(MAB11)	2												
22	VG5	PE-Cy5	CD3	(MAB11)	2												
23	VG8	PE-Fire 744	CD25	(1243)	1					1						1.2	7.2
24	VG9	PE-Fire 770	HLA-DR	(1243)	1												
25	VG10	PE-Fire 810	HLA-DR	(1243)	1												
26	R2	Alexa Fluor 647	CD39	(A1)	1							0.8	4.8				
27	R4	APC-R700	CD107a	(MO1-2)	1					1.2	7.2						
28	R6	Zombie NIR	L/D	(H4A3)	1.2	7.2										1.2	7.2
29	R7	APC-Fire 750	CD27	(3223)	2												
30	R8	APC-Fire 810	CCR7	(3223)	1							1.5	9				
And UNSTAINED CONTROLS !!!				Antibody Total	1.2					4.8							
				R10 Media	19.3					12.7	76.2	13.9	83.4			10.5	63
				Pipette draw volume	19.5					25.4	152.4	27.8	166.8			21	126
										35.1	38.7					28.5	

cpb T cell SFC panel

Wash with 2 ml PBS, spin down 1300rpm 8min  
800 ul of LiveDead mix (1:2500) @RT for 15min  
Wash 1.5 ml 5% PBS-FBS, spin 1300 rpm, 8min

Add CD161 biotin antibody for 10 minutes at RT

Add HoeStain mix, incubate @ 37C for 30min  
Wash 1.5 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 1.5 ml 5% PBS-FBS 1400 rpm, 6min

Add Streptavidin Mix, incubate @ 4C for 15min  
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min

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

First PermWash: 1.5 ml NuclearWash 1500 rpm 6 min

Second Perm Wash: 1.5 ml NuclearWash 1500 rpm 6 min

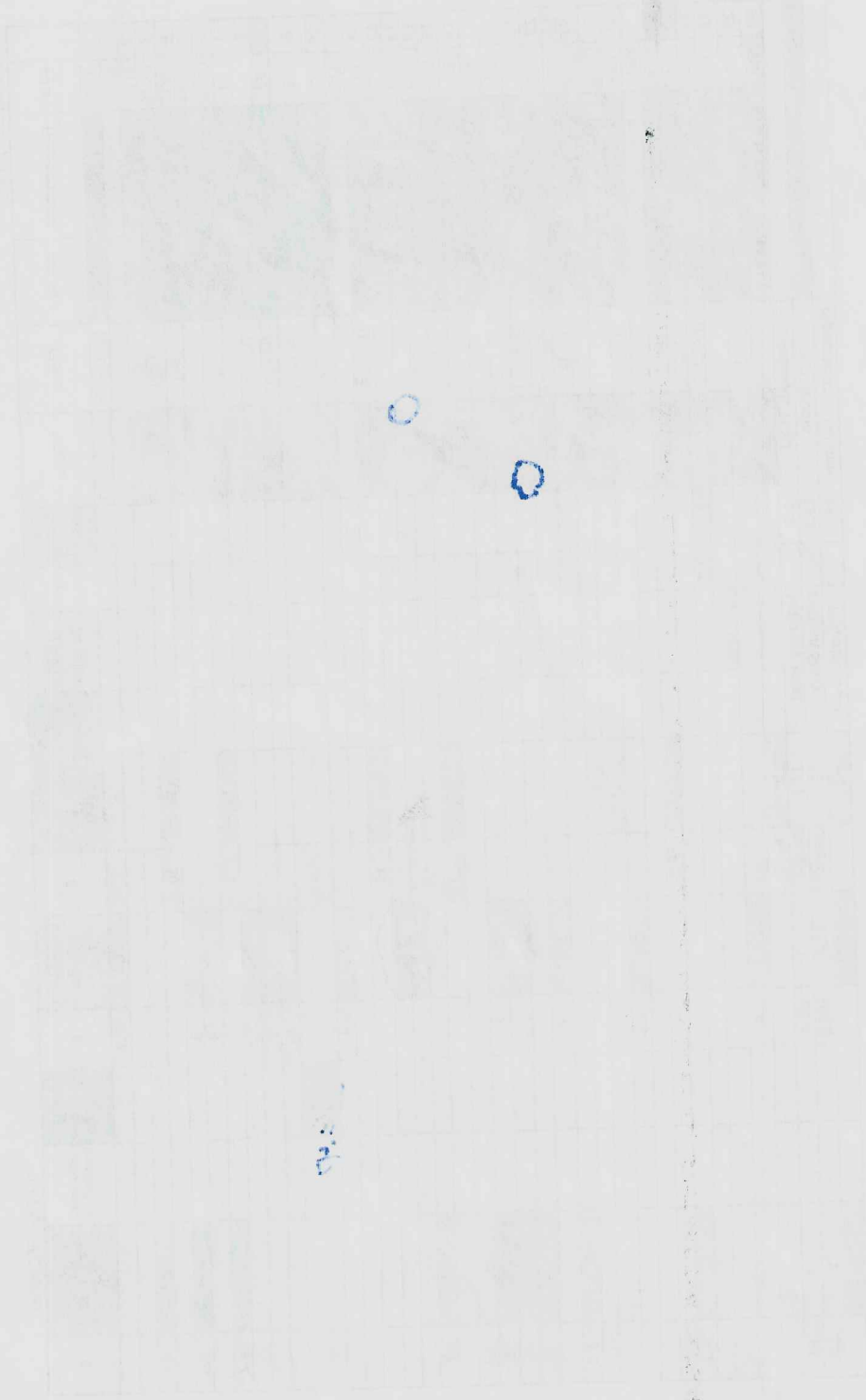
Add Intracellular Stain, incubate @ RT for 40min  
First PermWash: 1.5 ml NuclearWash 1500 rpm 6 min  
Second Perm Wash: 1.5 ml NuclearWash 1500 rpm 6 min

Resuspend in 80 ul 0.4% PFA-PBS  
Cap tubes, wrap rack in foil, store at 4°C

1005/P01  
C007  
C0025 - T<sub>H</sub>4  
C0023 - T<sub>H</sub>1-CT<sub>H</sub>4  
C006 -

T<sub>H</sub>1 = LAG3, CD279 but not CD23

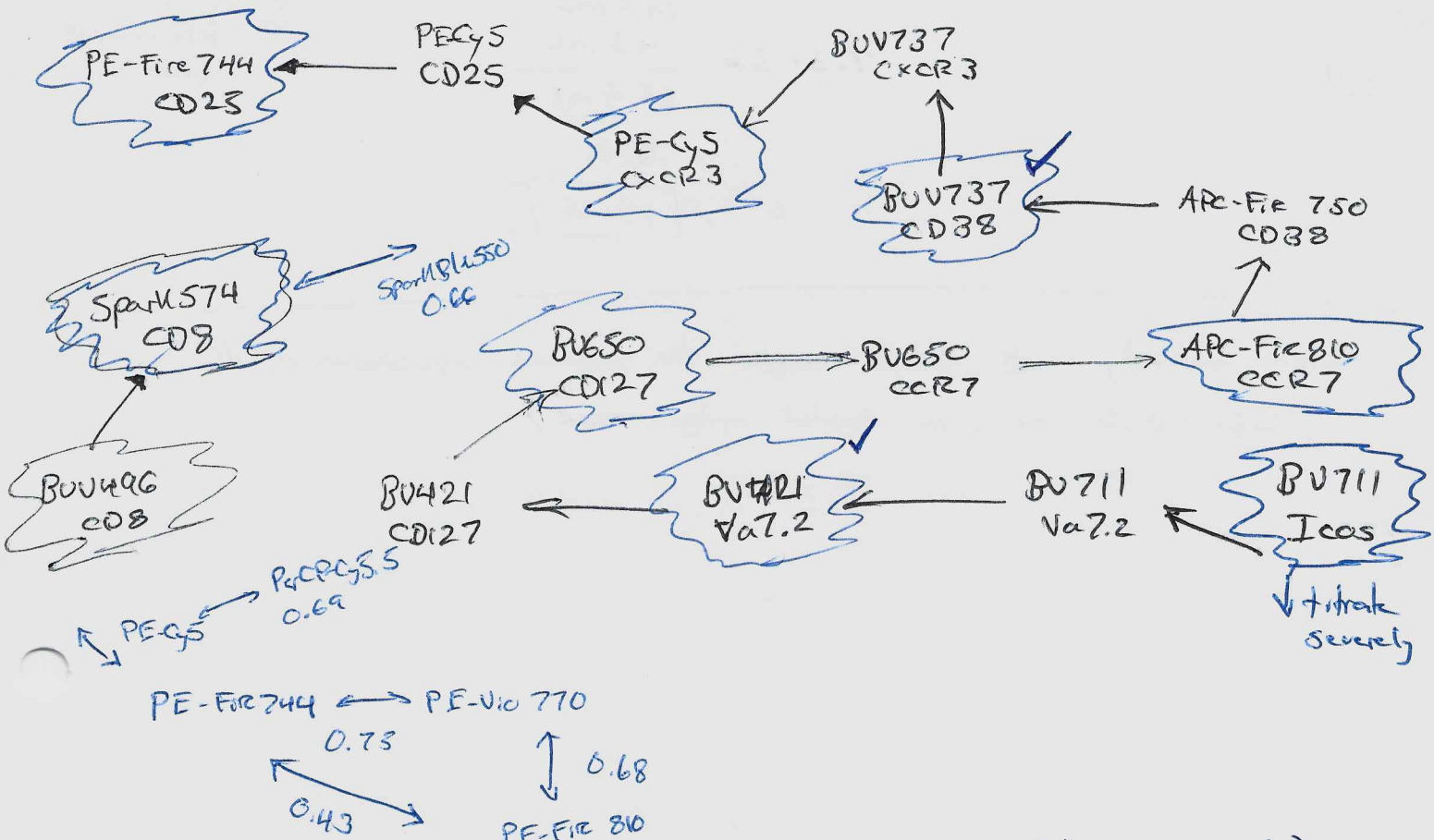
12.2 = 2.4  
32x2 = 6.4



15.5  
4.4  
4.0



RBX vs PerCP-Cy5.5 CD123 (for crisis)



Beads for each new floor

Set A  
Spark CD8  
APC-Fire810 CCR7  
baseline

4 (2)	4 (2)	6 (2)
BVU50 CD127 PE-Fire 744 PE-Cy5 CD25	BVU711 Icos PE-Cy5 CXCR3	All

12 units Act x 3 = 4.2  
 30 units stim x 3 = 9.6  
 30 units  
 5 wells ctrl

2 wells SEB

26.0  
13.8  
12.2 = 6 wells

0.67 0.33

25%  
SEB 1.2 M  
5 µL/ml CD47a  
10 µL/ml GdG

FBS/pBS:

5

$$6 * 4 * 1.5 = 36$$

$$48 * 1.5 = 72$$

108

7 extracted

2 vial

4 RBS pBS

$$6.25 * 1.5 * 4 =$$

$$48.75 * .750 * 2 =$$

&lt; 4

$$9 * 4 * .750 =$$

$$19.2 \text{ mL}$$

$$4.8 \text{ mL}$$

$$25.0 \text{ mL}$$

$$728 = 5.6$$

~~18.75~~

6.75

18.25~~30~~  
~~58.5~~  
~~27~~121.5FBS-wash  
pBS

when antibody  $\neq$  bright enough to surpass equilibrium cutoff?  
(pBS-pBS.5 or a limited antigen case)

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