Jan 24th, 2025

Specimen	Status	Location	Conc	Date	Notes	Volume	CD45+	Total	Resuspension
ND032									
Inf9144 a-1	4M UZ6 N								

24th
January 12th, 2025

(Cells * 1000) / Volume

..54 Thaw NBO 38 10 M July 21, 2005
11:56 Thaw Inf 914
11:59 spin
12:15 DNAse 30 µL
12:27 start for cook

UZ6K 914-4 4M a-1 Infant

7.8 Million ↑2.3
↑5.0 (6 accidentally)

7.8M
18M

Rest @ 1pm → 7pm

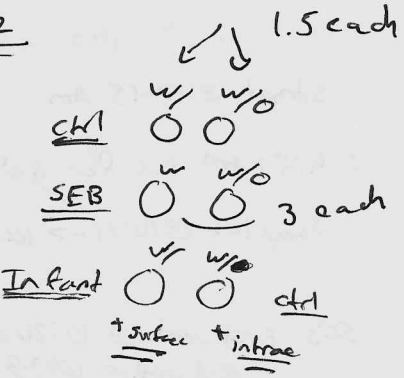
2.6E7 ↓
7.8E6

1.8 E7 1.8E7

$C_1V_1 = C_2V_2$

2.6(.3) =

1.5 each



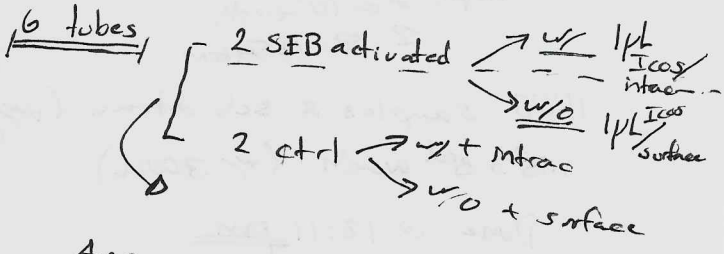
Meanwhile for Infant...

○ ctrl w/o @ surface ← See ICOS on constitutive circulating TFHs?

○ SEB w/o @ surface ← see upregulation after activation surface in absence of golgi plg

@ 7:08 SEB added
5.5 ml w/ Golgi 60 ml 3.3.20 → including sc's
1.2 or .6 µL CD107a to samples
9:08 pm Golgi Plg added

6 tubes



Answer: Do we see ↑ after activation that makes it to the surface

Q: Is there an accumulation after activation when golgi-blocked vs. the control)

Is the % Icos we see surface proportional to that intracellularly for both act/ctrl?

Is Icos held in reserve w/in ctrl specimen?

Is D in staining intra vs surface @ given amounts.

Aliquoting @ 7:08 AM

spin @ 7:23 AM

7:41 samples L/D spin 7:53

7:50 Hot sc's spin @ 8:25 AM

cold @ 8:02 am

Samples worked 8:09

Samples Hot @ 8:21 → 8:51 am spin @ 8:53

Doublet stopt to 0.8 µL/sample

All ABs prepped by 8:51 AM ✓

9:05 am Samples cold → 9:35 AM. Spin @ 9:35

stopt @ 9:45 AM

~ 9:51 AM Fix for sc's added ? → 10:01 → 10:11 → 10:21 AM

Samples @ 10:11 → 10:21 → 10:31 → 10:41

sc's First wash @ 10:26 am

2nd wash @ 10:38 am

Samples 1 @ 10:46 AM

2 @ 10:54 AM

11:08 samples & sc's intrace (w/ golgi's i can)

11:53 3rd wash (w/ 300µl)

Done @ 12:11 pm

SKyFeed

mcmc_stan

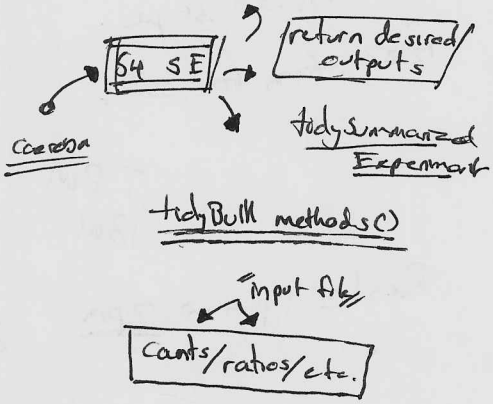
HMc

McMC

Stan

CmdStan rstan

mc-stan



XYZ % 30 → 60

$\frac{60}{30}$ = 2-fold

/30

Beckman Coulter

CytoFlex

Violet Blue Red

Fucas Celesta 405 488 640

↑ up to 14

Violet Blue Red