Sept 13, 2022

~~August 17th, 2022~~

Titration #2

~~PSEC Panels #1~~

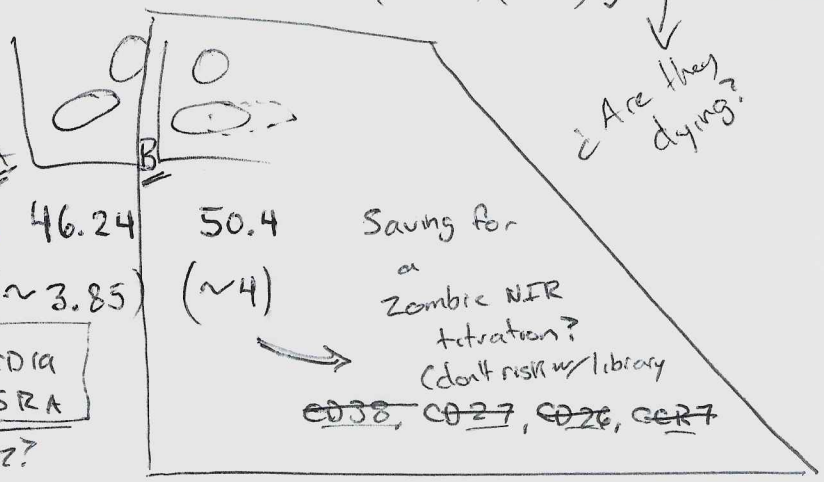
| | Specimen | Status | Location | Conc | Date | Tasks | Volume | Ly | Ly+Mon | Total | 1.5E+6 | 0.5 E+6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 240c/µl | NY057 (1) | adult | 12-10-15 | 2x $10^7$ | | | 4 | 3.81 | 4.74 | 15.24 | .787 | .262 |
| 230c/µl | NY057 (2) | adult | 12-10-15 | 2x $10^7$ | | | 4 | 3.80 | 4.67 | 15.2 | .789 | .263 |
| 240c/µl | NY057 (3) | adult | 12-10-15 | 2x $10^7$ | | ?DNase | 4 | 3.94 | 4.78 | 15.8 | .761 | .253 |
| | NY057 (4) | adult | 12-10-15 | 2x $10^7$ | | suffered ↓FSC | 4 | (4.25) | (~~4.80~~ 5.03) | 17.0 | .706 | .235 |

3:00 pm start batch 1

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | NY057 (5) | adult | 12-10-15 | 2x $10^7$ | ↓FSC | 4 | (3.93) | (4.66) | 15.7 | .763 | .254 |
| 380c/µl | NY057 (6) | adult | 12-10-15 | 2x $10^7$ | ↓FSC | 4 | (4.42) | (5.09) | 17.7 | .679 | .226 |

3:00 pm thaw start
3:53 pm SDS stain
4:08 count start

¿Are they dying?




Unstained 3M ~300µl (1.5M) for live spike ins

CD14, CD19
CD45RA
+CD127?

1ml PBS ~~FBS~~ 1500 RPM 8min
Spin @ 5:09 pm
FBS spin @ 5:22 pm (no FBS spin for L/D, just 2ml pbs) due to mop up potential
(60µl Brilliant stain) + residual + ab cocktail @ 5:32 start
5:50 incubation start → 6:20 pm
L/D spin downs @ 6:11
6:22 - 6:24 RBC lyse
→ 6:26 pm
L/D stain in @ 6:32 2ml 5% FBS-PBS
→ 6:37 pm → 6:52 pm
Fix Perm @ 6:41 PM → 51 → 7:01 pm
6:57 screwed up L/D by adding 1ml PermWash to every tube
Fuck!
proceeded add 2ml FBS + PBS & spin @ 1400 6min

"Just permibalized everything"

I fucked up... Saponin is added @ this step

~~August 17th, 2022~~

~~vs SFC Panels #1~~

100 µl DMSO + 2 vials NIR

100 test vials (1:100 – 1:1000) 1:5000 1:2500 → 2/5 ml

1-10 e^6 cells in 100 µl (no protein in PBS)

(15-30 min stain)

wash 2 ml w/ FBS

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| | | | | | |
|---|---|---|---|---|---|
| | | 1:5000 | 1 µl | | |
| | 100 | 200 | 400 | 800 | 1 ml |
| 3 million cells / tube | | 1:2500 | 2 µl | | |
| Unstained | 100 | 200 | 400 | 800 | 1 ml |
| | | 1:1000 | 5 µl | | |
| Unstained | 100 | 200 | 400 | 800 | 1 ml |

Spike in the "Live" NY057 aliquots

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want dye + saponin → introduction w/o being necrotic

Post-saponin 1 FBS Rm Wash + 2 ml FBS-PBS 1400 rpm 4 min

another + 2 ml FBS-PBS 1500 rpm 6 min

7:10 pm

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7:23 pm "Fix" for L/D

L/D "first" permwash (Zyrell) @ 7:46 pm

Done @ 8:13 pm

~ 5 hour process

~~August 17th 2022~~

~~A SFC Batch #1~~

12:28pm start

sitflush unstained to get record ✓
adjusted FS ↓ a tad
NY057 healthy cells look healthy.

1:09 pm ~ acting clotty ↓↑ in rate
by about 1000 cuts

Zanhe NIR → collecting scatter
2500 100/200 <
400, 800 >0

result 6% of the adm?
macrophages look fine though...

1:5000 unstained event rate
↑6900 after sitflush ~500?
& vortex
2µl/min vs 20µl/min

sample quality ~ stalling.

Done @ 2:18pm