

N=190 Apt: 10:30 AM - 12:00 PM

January 5-8th, 2021

HEU: GolgiStop-Plug Concentration

18 hours rest, 2 hours stimulation, 18 hours incubation;
0.5 µg/ml Costim (αCD28/CD49d) & 10 µg/ml TTX

Specimen	Status	Location	Date	Tasks	Volume	Ly	Ly+Mon	Total	↑ 1M/ml
ND050 #1	Adult		10/21/20		3 ml	2.25 E4	3.41 E4	6.75	3.75
ND050 #2	Adult		10/21/20		3 ml	2.01 E4	3.05 E4	6.03	3.03

Testing for #2 exp determined purposes

Adult 1.0E+6 cells	Ex vivo	Extracellular *6.5	Intracellular *6.5
V450 BV421	CD69	1	6.5
V525 BV510	Aqua	1:500	-
V670 BV650	CD4	1.8	11.7
B530 FITC	CD8	1.5	9.75
B710	CD8	0.5	3.25
PerCPeF710	TNFA	-	19.5
Y590 PE	CD62L	2	13.0
Y780 PE Cy7	IFNg	-	5.2
R670 APC	CD3	1.5	9.75
R780 APC-Fire750	CD45RA	1.5	9.75
PBS		11.2	72.8

3 ml

3.0E4 3.9E4 9.80E4 9.7E4
starting late evening to avoid a R 10:00 AM Seminar. Will see if successful.

Tuesday 8:16 pm: Thaw start 9/12.78 20.25

8:40 pm: PE staining
9:02 pm: Done counting, Ever clean start

13 M cells @ Incubation: 9:12 pm →
+ 18 hours: [Weds @ 3:12 pm]

500 µl 1 ml
25 5 10 15 20
1 2 3 4 5
1 2 3 4 5

Cell Cone → [2M/ml concentration]

5 µg/ml of TT @ 500 µl ~ 10 µg/ml (1.785 µl)

10 µg/ml of TT @ 1500 µl ~ 10 µg/ml (3.57 µl)

Costim @ 0.5 µg/ml each 5 µl + 5 µl + 42 µl = [5 µl / 1%] @ 1 ml
→ 0.5 µl + 0.5 µl

GolgiBlock/stop 1:1:19 = (10) @ 1 ml

800 µl
4800
Aqua 10 µl = 5 ml
18 ml FBS-PBS → (10 × 2)
20 ml Bm wash (2-18)

(1/2th) ~ 0.5 µg/ml

1:1:48 = (10) (1/5th) ~ 0.2 µg/ml

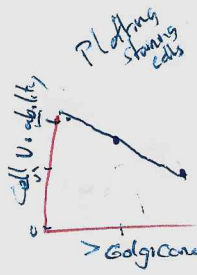
500 µl:
(effective) 1/2 (0.25 µg) 1x (~0.5) 2x (1 µg)
[0.125 µg] [0.25] [0.5 µg]

1000 µl
1/4 1/2 1x
0.125 µg 0.25 µg 0.5 µg
K shouldn't retain well.

Not gating

No cold rinses appeared to work well, lots APCs on stain

Save Supernatants



"col" Weds: 3:40 pm Stain count started

280 cells/µl. 3 ml * 3 E4 = 9 M cells
LTM 3.9 E4 ← Pretty good

13 M scy 6 million cells.

4.5 ml ↑ 2M/ml

Inc start @ 16:14 pm { 18:17 pm + 18 hours

SC start: 5:29 pm
5:45 pm SC stain Thursday

SC's done @ 6:27 pm Inc Done ✓

12:13 pm spin for supernatants

12:44 pm L/D

13:14 pm SC in

Fix Perm @ 13:47 pm

Post Perm wash 2:10 pm

Intracellular scs @ 2:32

Done @ 3:27 pm

Am I getting too high on CD83 / missing response that way?

500 μ l 25 ~~000~~ CD69 expression few cytotline cells (prob low gp5/p)

I forgot my VSB! (crying face).

- more IFN γ producing cells?

1.3 mg/ml

10,000 PD1 + 10,000 PD2

10,000 PD1 10,000 unselected

TCR plate same # beads coated uplaid 20,000 unselected

TCR + PD1 & (plate) (small)
~ PD1 & soluble. (not neutralized)

125 pg

+ PD1 pdt free, no Δ .
Die on through culture?

Die no PD1 (not @ PD2 low MZ)
A weak read as was.

not PD1 engagement
PD1 / PD2 PBMCs PD1 \uparrow less pattern
Hyp:

dg9 PD1. 55 pg
neg
cyls not + cytotline bulk