

February 11-13th, 2021

HEU timecourse: Rest optimization #2

12, 14, 16, 18 hrs rest

2+18 stimulation w/golgi

0.5 µg/ml Costim (αCD28/CD49d) [2.5ul] & 10 µg/ml TTx, DTx

GolgiStop-Plug 1:1:19 (5)

260 c/pl

300 c/pl

240 c/pl

| Specimen | Status | Location | Date | Tasks | Volume | Ly | Ly+Mon | Total | | |
|----------|--------------|----------|------|---------|--------|------|--------|-------|-------|------------|
| ND050 | Adult DTT | | 10EG | 10/2/20 | 3 | 2.09 | 3.16 | 6.27 | ↑3.27 | ÷2 = 3.13 |
| ND050 | Adult DT | | 10EG | 10/2/20 | 3 | 2.35 | 3.50 | 7.05 | ↑4.00 | ÷2 = 3.50 |
| ND006 | Adult SEB | | 20EG | 7/8/19 | 6 | 3.25 | 3.95 | 19.50 | ↑13.5 | ÷4 = 4.875 |

| Adult 1.0E+6 cells | Ex vivo | Extracellular x18 | | | Intracellular x18 | |
|-----------------------|---------|----------------------|-------|--------|----------------------|-------|
| V450 BV421 | CD69 | 1 | 18.1 | CD69 | 1 | 18.15 |
| V525 BV510 | Aqua | 1:500 | - | Aqua | - | - |
| V670 BV650 | CD4 | 1.8 | 32.4 | CD4 | 0.5 | 9 |
| B530 FITC | CD8 | 1.5 | 27 | CD8 | 0.5 | 9 |
| B710 | TNFA | - | - | TNFA | 3.0 | 54.0 |
| PerCPeF710 | | | | | | |
| Y590 PE | CD62L | 2 | 36 | CD62L | - | - |
| Y780 PE Cy7 | IFNg | - | - | IFNg | 0.8 | 14.4 |
| R670 APC | CD3 | 1.5 | 27 | CD3 | 0.5 | 9 |
| R780 APC-Fire750 | CD45RA | 1.5 | 27 | CD45RA | - | - |
| PBS | | 11.2 | 201.6 | | 14.2 | 255.6 |

8:06 last ND006 Hare

8:08 spin

8:28 Se incubation

8:40 constant

combined both ND050s - grating happy
distributed 3.25 across

Plated @ 9:07

20 tubes
x 7 min

140
2

LHC 7.16

add CD3 FITC
outd CD3 APC
both set mine
7:38 Edore

Starting Salento Hood @ 9:20 am

9:36 am spin on Merck's

9:48 am out.

10:02

Cent @ 10:13 am

Plated @ 10:47 am

20 samples

x.8
16.0 ml
~32 pl 4/0 Aqua

60 mls FBS-PBS

60 mls PermWash
6 ml into 54 ml

Resuspend into (250 pl) & (200) this time.

8.8
3ml

5.8ml

20 x .3 = 6.0 20 x .2 = 4.0 mls ✓

PFA-PBS ✓

Saving supernatants?

05/01

05/02

05/03

05/04 12/1 (05)
05/05 12/2 (05)

05/06

05/07

05/08

05/09

05/10

05/11

05/12

05/13

05/14

05/15

05/16

05/17

05/18

05/19

05/20

05/21

05/22 (05/22) 12/2 (05)

05/23

05/24 12/3 (05)

05/25

05/26

05/27

05/28

05/29

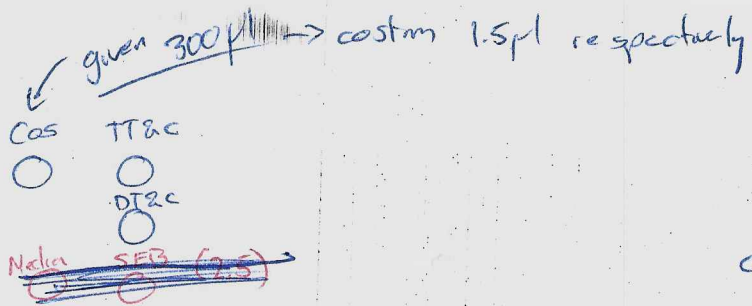
05/30

05/31

06/01 12/4 (05)

06/02 12/5 (05)

06/03 12/6 (05)



can't messd. ups.
 All event *
 S.P.OI. RI

| 12 hrs rest: | Projected | Actual |
|--------------|-----------------------|-------------------------|
| Thaw: | | 8:00pm |
| Count: | | 8:40pm |
| Plate: | | 9:07pm |
| Process: | 9:10 am | 9:20 am |
| Count: | | 10:13 |
| Stim: | | 10:47 |
| Golgi: | 12:47 | 12:47 |
| Spin: | 6:37am | |
| Spin2: | 6:55 AM \rightarrow | |
| L/D: | | 7:16 |
| Extra: | | 7:38 \rightarrow SB |
| Intra: | | 8:53 \rightarrow 9:33 |
| End: | | 9:43 |

but back no setting up

300 μ l / 300 μ l

170 μ l

count ND050

1 ml

count ND006

2 ml

Mostly dead?

L L+M Total
2.59E4 3.60E4

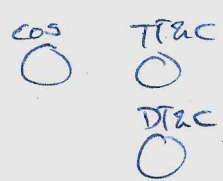
Dead

500 μ l aliquot after fact showed all/almost all dead by microscope

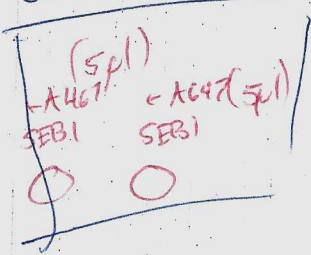
First Perm Wash @ 8:36 am (10 minutes later)

200 μ l ~ 1 μ l costm

CD8 degranulation: Panel A+B



Media SEB (2.5)



Microscope visualization says all are

11:58 am count 12:07 finally got Guava to show cells

L L+M Total

310 μ l ND050

1 ml

2.4

2.99E4 \rightarrow 1.2

340 μ l ND006

1 ml

3.9

4.36E4 \rightarrow 1.95

12:29 TT in \rightarrow 12:35 SEB in / vortexed " / 12:35 rotate

(Delay until 2nd perm wash is out for 12)

Start @ 8:45 (9 min late)

Fix Perm @ 10:07

First wash @ 10:30

| 14 hrs rest: | Projected | Actual |
|--------------|-----------|---------------------------|
| Thaw: | | 8:02pm |
| Count: | | 8:40pm |
| Plate: | | 9:07pm |
| Process: | 11:10am | 11:12am |
| Count: | | |
| Stim: | | 12:35 |
| Golgi: | 2:35pm | 2:36 |
| Spin: | 8:21am | |
| Spin2: | | |
| L/D: | | 9:02 \rightarrow 9:17 |
| Extra: | | 9:32 \rightarrow 9:52 |
| Intra: | | 10:50 \rightarrow 11:30 |
| End: | | 11:44 |

| 16 hrs rest: | Projected | Actual |
|--------------|-----------|---------------|
| Thaw: | | |
| Count: | | |
| Plate: | | |
| Process: | | 1:10 pm |
| Count: | | 1:51 pm |
| Stim: | | 2:25 pm |
| Golgi: | 4:25 pm | 4:25 pm |
| Spin: | 10:25 am | 10:18 |
| Spin2: | | |
| L/D: | | 10:28 → 10:43 |
| Extra: | | 10:58 → 11:18 |
| Intra: | | 12:09 → 12:49 |
| End: | | 1:00 pm |

Friday

Saturday

13:15 start (CD24 ab arrived)

Used R10 instead of old RPMI II

Spin @ 1:28 pm

13:48 done process

stain count @ 1:51 pm

Forgot to check 1000 counts (1200) (1400)

11:30 c/f Fa Perm wt

first perm → 11:11 am
2nd @ 12:00

| | Ly | Ly+M | Total |
|---------------------------------|------|------|-------|
| 1 ml | 1.7 | 2.17 | |
| 1 ml | 3.19 | 3.63 | |
| 1.7 / 3 = .630K → Single colors | | | |

Need train by 3:45

| 18 hrs rest: | Projected | Actual |
|--------------|-----------|-----------------|
| Thaw: | | |
| Count: | | |
| Plate: | | |
| Process: | 3:02 | 3:10 pm |
| Count: | | 3:49 → 59 |
| Stim: | | 4:12 pm |
| Golgi: | 6:12 pm | |
| Spin: | 12:12 pm | 12:07 |
| Spin2: | | |
| L/D: | | 12:17 → 12:32 |
| Extra: | | 12:45 → 1:05 pm |
| Intra: | | 2:01 → 2:41 |
| End: | | 3:57 |

Friday

Saturday

Cos 620u
TTRC 620u
DTAC 620u

Media SEB (2.5)

Spin @ 15:14 pm

stain @ 15:35 pm

3:49 pm backflushed tried again to get grown to warm post clean.

180 c/p ND050

270 c/p ND006

| Ml | Ly | Ly+M | Total |
|----|------|------|--------|
| 1 | 2.02 | 2.45 | → 670K |
| 1 | 3.43 | 3.81 | → 1.90 |

Just made unnecessary FBS-FBS II why?! 1:09
Fax Perm @ 1:21

Forje down

scb @ 3:02 → 3:27
(10 min under pre)

16 hr stim
w/gdgi
w/5M (15M) → (10M)

18 hrs 6 am
12 pm → 4
5 → 8



Side Experimental Gigs:

APC hCD1d 1.3 mg/ml
1.3 µg/ul

NKT test: x2

Done @ 9:12 am

| hrs Stimt: | Projected | Actual |
|------------|-----------|--------|
| Thaw: | | |
| Count: | | |
| Plate: | | |
| Process: | | |
| Count: | | |
| Stim: | | |
| Golgi: | | |
| Spin: | | |
| Spin2: | | |
| L/D: | | |
| Extra: | | |
| Intra: | | |
| End: | | |

| | | | | | |
|----------|----------|--------|------|------|------|
| BV421 | CD4 | 1.8? | 3.6 | 0.5? | 1.0 |
| BV510 | 1/2 Aqua | 1.500 | — | | |
| BV650 | CD56 | 1.8 | 3.6 | | |
| Fitc | dg9 | — | — | 3 | 6.0 |
| PerCp | CD3 | 1.0 | 2.0 | 0.2 | 0.4 |
| Y590 | NKG2D | 0.6 | 1.2 | | |
| Y780 | NKG2A | 0.6 | 1.2 | | |
| APC | cd1d | 1.3 µl | 2.6 | | |
| APC-Fitc | CD8 | 1.5? | 3.0 | 0.5? | 1.0 |
| PB 5 | | 11.9 | 23.8 | 16.3 | 32.6 |

ND006 ○

± 5 µl pipette overfill?

★ If my IL-4 shows, swap it in vs IFNγ for a TTK/DTx 18hr well. ★

Done @ 8:33 am ✓

A: TNFα

CD8 Test degranulation

CSEB 2.5 µg/ml

B: IFNγ

| | | | | | |
|----------|----------|-------|------|------|-----------|
| BV421 | CD69 | 0.5 | | 1 | |
| BV510 | 1/2 Aqua | 1.500 | | | |
| BV650 | CD4 | 1.8 | | 0.5 | |
| Fitc | CD107A | <5> | ~10? | | |
| PerCp | TNFα | — | — | 3.0 | |
| Y590 | CD62L | 2.0 | | | |
| Y780 | CD8 | 1.5? | | 0.3 | |
| APC | CD3 | 1.5? | ✓ | 0.5? | None left |
| APC Fitc | CD45RA | 1.5 | | | |
| PB 5 | | 11.7 | | 15.0 | |

CD8
PerCp 7
Stim
Beam

ND006 ○

| | | | | | |
|----------|----------|-------|------|------|---|
| BV421 | CD69 | 0.5 | | 1 | |
| BV510 | 1/2 Aqua | 1.500 | | | |
| BV650 | CD4 | 1.8 | | 0.5 | |
| Fitc | CD107 | <5> | ~10? | | |
| PerCp | CD3 | 1.5? | | 0.5? | ✓ |
| Y590 | CD62L | 2.0 | | | |
| Y780 | IFNγ | — | — | 0.8 | |
| APC | CD8 | 1.5? | | 0.5? | ✓ |
| APC Fitc | CD45RA | 1.5 | | | |
| PB 5 | | 11.7 | | 17.2 | |

ND006 ○

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Degranulation/NKT tetramer tests

| Degranulation TNF α | Ex vivo A β T cells | Surface μ L x | | Intra μ L x | |
|-------------------------------|------------------------------|------------------------|---|--------------------|--|
| V450 BV421 | CD69 | 0.5 | | 1 | |
| V525 BV510 | Aqua L/D | 1:500 | | | |
| V670 BV650 | CD4 | 1.8 | | 0.5 | |
| B530 FITC | CD107A | <3ul added separately> | | | |
| B710 PerCPeF710 | TNF α | - | - | 3 | |
| Y590 PE | CD62L | 2 | | | |
| Y615 PE Dazzle | | | | | |
| Y780 PE Vio770 | CD8 | 1.5 | | 0.5 | |
| R670 APC | CD3 | 1.5 | | 0.5 | |
| R780 APC Fire750 | CD45RA | 1.5 | | | |
| 20.5 μ l/rxn; | PBS | 11.3 | | 15 | |

| Degranulation IFN γ | Ex vivo A β T cells | Surface μ L x | | Intra μ L x | |
|-------------------------------|------------------------------|-------------------------|---|--------------------|--|
| V450 BV421 | CD69 | 0.5 | | 1 | |
| V525 BV510 | Aqua L/D | 1:500 | | | |
| V670 BV650 | CD4 | 1.8 | | 0.5 | |
| B530 FITC | CD107A | <3 ul added separately> | | | |
| B710 PerCPeF710 | CD3 | 1.5 | | 0.5 | |
| Y590 PE | CD62L | 2 | | | |
| Y615 PE Dazzle | | | | | |
| Y780 PE Vio770 | IFN γ | - | - | 0.8 | |
| R670 APC | CD8 | 1.5 | | 0.5 | |
| R780 APC Fire750 | CD45RA | 1.5 | | | |
| 20.5 μ l/rxn; | PBS | 11.7 | | 17.2 | |

| Intracellular Test | NKT | Surface μ L x | | Intra μ L x | |
|-----------------------|-------|----------------------|---|--------------------|--|
| BV421 | CD4 | 1.8 | | 0.5 | |
| BV510 | L/D | 1:500 | | | |
| BV650 | CD56 | 1.8 | | | |
| FITC | dg9 | | | 3 | |
| PerCPeF710 | CD3 | 1 | | 0.2 | |
| PE | NKG2D | 0.6 | | | |
| PE Dazzle | | | | | |
| PE Vio770 | NKG2A | 0.6 | - | | |
| Alexa 647 (APC) | hCD1d | 0.6 | - | | |
| APC Fire750 | CD8 | 1.5 | | 0.5 | |
| 20.5 μ l/rxn; | PBS | 12.6 | | 16.3 | |

