

February 5-8th, 2021

HEU: Timecourse Stim Optimization

18 hours rest, 2 hours stimulation,

3, 9, 15, 21, w/golgi

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

GolgiStop-Plug 1:1:19 (5)

Specimen	Status	Location		Date	Tasks	Volume	Ly	Ly+Mon	Total	~time	
ND050	Adult T _H 1		10EG	10/21/20		3	2.03	2.91	6.09	4.3	
						2	2.71	3.44	5.42	—	2.71 ↑ 710 µl
C4282	Adult PPD		10EG*	10/12/20		3	1.70	1.98	5.10	3.6	
						1.5	2.30	2.60	3.45	—	1.723 ↑ 225 µl
ND006	SEB ? DTx		15EG	3/13/20		5	3.33	4.15	16.65	11.7	
	clump					5	2.23	2.61	11.15	—	5.85 ↑ 850 µl
ND050	Adult T _H 1		10EG	10/21/20		3	2.61	3.91	7.88	5.4	
						2	2.77	3.40	5.54	—	2.77 ↑ 770 µl
C4282	Adult PPD		10EG*	10/12/20		3	2.02	2.50	6.06	4.2	
						1.5	3.37	3.71*	5.05	—	2.52 ↑ 1000 µl
ND006	SEB ? DTx		15EG	3/13/20		5	2.91	3.68	14.55	10.2	
						5	2.75	3.20	13.75	—	6.875 ↑ 1.875 µl

★ Using up last FBS-PBS from January ★

Adult 1.0E+6 cells	Ex vivo	Extracellular x 24		Intracellular x 24
V450 BV421	CD69	1 24	CD69	1 24
V525 BV510	Aqua	1:500 —	Aqua	— —
V670 BV650	CD4	1.8 43.2	CD4	0.5 12
B530 FITC	CD8	1.5 36	CD8	0.5 12
B710	TNFA	— —	TNFA	3.0 72
PerCPeF710				
Y590 PE	CD62L	2 48	CD62L	— —
Y780 PE Cy7	IFNg	— —	IFNg	0.8 19.2
R670 APC	CD3	1.5 36	CD3	0.5 12
R780 APC-Fire750	CD45RA	1.5 36	CD45RA	— —
PBS		11.2 268.8		14.2 340.8

Make 2 separate
times 12-1
& 6-7

68.8 µl
4/0 hzm
34.4 ml

Core @ 2M/ml so 500 µl = 1M cells

Ttx old = 1.785 µl = 5 µg ~ 10 µg/ml (3.57 µl in 1 ml situations)

DTx = 0.5 µl stock = 5 µg ~ 10 µg/ml (1.0 µl in 1 ml)

Costim:

5 + 5 + 42 = 5 µl ~ 1 ml

2.5 µl ~ 250 µl

Golgi:

1 + 1 + 19 = (5) for 500 µl
10 for 1 ml

PPD 1 mg/ml so 1 µg/1 µl ~ 5 µl for 500 µl
~ 10 µl for 1000 µl

SEB (10 µg/ml) 1 mg/ml so 5 µl ~ 10 µg/ml

New T_H test 1:1:19

3.85 µl for 500 µl
7.70 µl for 1000 µl

2nd batch:

5:44 → 6:14 ✓ out plate ✓
clump on ND006

10.0 = 5
7.5 = 3.75
5.0 = 2.5

6:50 into 15 ml
6:50 stain start
Count start: 7:01 AM
Done: 7:12 AM

8:51 start
Ready: 7:38 AM
Cost: 7:40
Ant: 7:45
SEB: 7:46
Finished 1.45 SEB
incubation start

3 9 15 18 21
1st batch: 9
2nd batch: 15
Not post
only issue resolution

Processed: 5:43 → 6:12 pm ✓ out plate
6:40 pm into 15 ml

stain start 6:42 pm
count start 6:53 pm
8:04 pm done

7:45 costim
7:53 antigens
7:58 SEB
7:57 incubation
start

3 hrs Stim:	Projected	Actual
Thaw:		10:46
Count:		11:26
Plate:		11:48
Process:	5:48 AM	5:43 → 5:40
Count:		7:12
Stim:		8:51 AM
Golgi:	9:40 am	9:52 AM
Spin:	12:42 pm	12:43
Spin2:		1:10
L/D:		1:26 pm
Extra:		2:05 pm
Intra:		15:38
End:		4:37

Saturday

Sunday

Supermarket
shopping →

costim

8000c?
costim

Media

costim

TT+c

PPD+c

SEB 10

costim

Excess DT+c (1/2)

SEB

SEB

FBS wash @ 13:48 pm

Last FBS wash @ 14:39 pm

Golgi @ 2:46 pm, 5b

1st F.m @ 15:10 pm 2^o @ 15:23



15 hrs Stim:	Projected	Actual
Thaw:		22:45
Count:		23:32
Plate:		23:53
Process:	17:53	17:43 → 18:40
Count:		19:04
Stim:		7:57
Golgi:	9:57 pm	9:50 pm
Spin:	12:50 pm	12:43
Spin2:	1:10 pm	1:10
L/D:		13:26
Extra:		14:05
Intra:		15:38
End:		4:37

Friday

Saturday
2 hrs

Sunday

costim

costim

Media

costim

TT+c

DT+c

PPD+c

SEB 10

DT+c

TT+c

PPD solo

SEB 7.5

SEB 2.5



22 tubes

2pm
4pm

Saturday

Sunday

9 hrs Stim:	Projected	Actual
Thaw:		10:46
Count:		11:26
Plate:		11:48
Process:	5:48 AM	5:43 → 6:40
Count:		7:12
Stim:		7:51
Golgi:	9:40 am	9:52
Spin:	18:12 pm	
Spin2:		7:07 pm
L/D:		7:22 pm → 7:37
Extra:		8:00 → 8:20
Intra:		9:35 → 10:15
End:	18:25	22:35 ✓

Costim TT+c
○ ○
○ DT+c

700k Costim PPD+c
○ ○

Media SEB¹⁰ 2.5 SEB¹⁰
○ ○ ○ ○
costim costim DT

(maybe up to 2M more)
extra priority to 9 hr DT+c

C might be a bit tight, up to 1.1 more)
distribute equally all likelihood

Cup to 7.7 M more)
SD's: 3M + Costim + DT+c well
4M
In rarely excess, TT+c well
@ 9, then 3.

Sunday 5:44 start process
6:11 am process spin
1/2 DFBJ wash @ 7:43 pm
100% FBS wash @ 20:25
Golgi G/FixPerm @ 8:40
First permeabilise @ 9:06

10 minutes ahead now

Friday

Saturday
2 hrs

Sunday

21 hrs Stim:	Projected	Actual
Thaw:		22:45
Count:		23:32
Plate:		23:53
Process:	17:53	17:43 → 18:40
Count:		19:04
Stim:		7:57
Golgi:	9:57 pm	9:50 pm
Spin:	18:18:30	
Spin2:		7:07 pm
L/D:		
Extra:		
Intra:		
End:		

Costim TT+c
○ ○
○ DT+c

Costim PPD+c
○ ○
Media SEB¹⁰ SEB^{7.5} SEB^{2.5}
○ ○ ○
Costim DT+c
○ ○
TT+c

Cup to 3.88 M more)
Do a DT+c @ 15 hours, then 21hrs

Cup to 2 M more)
Unlikely to yield excess, maybe
a PPD solo well @ 15hrs?

Cup to 8.2 M more)
test costim + DT+c for
both timepoints
Excess? into a TT+c well

Extra Excess SEB @ 5hrs?

414 pL

8 pm

22
x 800
17600

21 tubes
x 8
168

17.6
16.6
34.4 → 68.8 pL

2
43 tubes
x 7
301/60 5 hours

301
- 112
189
4
16
x 7
112

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

6 costim solo

4 ft
4 dt
3 ppcl

2 media

6 SEB

$$\begin{array}{r} 18 \\ \times 5 \\ \hline 90 \end{array}$$

25 samples
5 pl
125 pl golgi

$$\begin{array}{r} 4 \\ 19 \\ \times 5 \\ \hline 95 \end{array}$$

$$\begin{array}{r} 7.7: 19 \\ \times 7 \\ \hline 133 \end{array}$$

First golgi run

$$\begin{array}{r} 8:8:19 \\ \times 8 \\ \hline 152 \end{array}$$

5:5:45

8:04 sc start.

8:23 Incubation → 8:43

also 9:02 - sc's done, golgi block made

Flow: ran at d time, ran high

stim3 SEB Media, 7.5 x 5

stim9 SEB 7.5 x 5

15000 others, 100,000 SEB

Buazil's luggage, PE →

$$1.3 \text{ mg} = 1 \text{ ml}$$

$$1.3 \text{ mg} = 1000 \mu\text{l}$$

$$1300 \mu\text{g} = 1000 \mu\text{l}$$

$$\begin{array}{r} \text{old TI:} \\ 2.8 \overline{) 1.785} \end{array}$$

$$\begin{array}{r} \text{New Prep} \\ 1.3 \overline{) 5} \end{array}$$

3.846 pl

$$\begin{array}{r} 21 \\ 4 \\ 36 \\ \times 7 \\ \hline 252 \end{array}$$

~ over 4 hours
CO max blues

degradation → shift on ESC/sec?
as signal not as fr
as vesicle loss?