

23-25th  
January 10-13th, 2021

# HEU: Rest Optimization

0, 6, 12, 18, 24 hours rest  
2 hours stimulation, 18 hours incubation;  
0.5 µg/ml Costim (αCD28/CD49d) & 10 µg/ml TTx  
GolgiStop-Plug 1:1:19 (1)

Specimen	Status	Location		Date	Tasks	Volume	Ly	Ly+Mon	Total	Pinl	
260 µl ND050	Adult TT	-180°C	10E/6	10/21/20	Media, Cos, TT, TTx	3ml	2.06	3.21	6.18	140 µl	
250 µl ND050 scs	Adult TT	-180°C	10E/6	10/21/20	DT 2c, PPD & Cos	3ml	2.12	3.18	6.36	471 µl	180 µl
240 µl ND050	Adult TT	-180°C	10E/6	10/21/20		3ml	2.83	4.03	8.49	549	
230 µl cold wash					Media, Cos, TT, TTx, DT 2c, PPD & Cos	2ml	2.92	3.92	5.84	↑ 920 µl	
270 µl ND050	Adult TT	-180°C	10E/6	10/21/20	Media, Cos, TT, TTx, DT 2c & PPD partial	3ml	2.54	3.75	7.62	4.62	
50 µl						2ml	2.64	3.54	5.28	↑ 640 µl	
230 µl ND050	Adult TT	-180°C	10E/6	10/21/20		3ml	2.14	3.18	6.42	↑ 342	
270 µl Combined					4.46 - 6.56 = 13.46, 13.5	3ml	3.06	4.17	4.18	↑ 1.59	
270 µl ND050	Adult TT	-180°C	10E/6	10/21/20		3ml	2.42	3.56	7.26	↑ 4.26	
200 µl ND050 p2						2ml	2.41	3.00	4.82	↑ 410 µl	
250 µl						3ml	2.34	3.38	7.02	↑ 4.02	

Adult 1.0E+6 cells	Ex vivo	Extracellular		Intracellular
V450 BV421	CD69	1	2017	CD69 1 2017
V525 BV510	Aqua	1:500		Aqua -
V670 BV650	CD4	1.8	36	CD4 0.5 1085
B530 FITC	CD8	1.5	30	CD8 0.5 1085
B710 PerCPeF710	TNFA	-		TNFA 3.0 6051
Y590 PE	CD62L	2	40	CD62L -
Y780 PE Cy7	IFNg	-		IFNg 0.8 16136
R670 APC	CD3	1.5	30	CD3 0.5 1085
R780 APC-Fire750	CD45RA	1.5	30	CD45RA -
PBS		11.2	224	14.2 284 2414

\* Conc Final @ 2N/ml so 500 µl - 1ml  
TTx = 1.785 µg = 5 µg ~ 10 µg/ml (3.57 for 1ml)  
DTx = 0.5 µg stock = 5 µg ~ 10 µg/ml (1 µl for 1ml)

Costim:  
5+5+42 = 5 µl for 1ml  
2.5 µl for 500 µl

Golgi  
1+1+19 = 10 µl for 1  
5 µl for 500 µl

PPD @ 1mg/ml so 1 µg/1 µl so 5 µl

Yikes... a lot of  
fluorescence

8:26 AM Thaw  
8:46 AM first count incubation  
9:01 AM → 9:04 AM small clean cycle length  
containing 106 AM  
Incubator @ 9:13 AM [for 6, 12 hrs]  
9:16 AM → 9:34 AM deep clean cycle length  
~ 18 min clean cycle

6 hr rest stain @ 15:38 pm  
Inc @ 16:02 pm

Last Thaw set: 4:12 pm  
18 pl liquid transfer smoothness  
centrifuge @ 16:24 pm  
@ 16:48 pm stain

\* Bg bbb trending dead \*  
Inc @ 5:29 pm  
Plates Inc @ 5:35 pm

12 hr: 3rd plate @ 9:03 pm  
220 pm spin  
9:37 pm → stain count  
Inc @ 22:15 pm

\* Use new golgi block: spin all fluorescence for 3 minutes \*

18  
x 8 15ml → 30 µl L/O Aqua (make new batch)  
14400 18x3 = 54 ml FBS/PBS (25-25)  
54 ml Penicillin & make closer to time

Flow: 6:30 pm start analysis

8:30 sec (7 min max as our cutoff)  
2000+ → 700-800 cells/s

0 hour: TT2C > TT 7 coding as it's running  
Keeping consistent.

TRC & TRD

7:24 pm - weird green screen popped up

6 hr - lot mess in media only, coding clean up a bit?

DTK - lot more NR spike at activation time, TNR, then w/ TR.

beats! responding PPD calls.

12 hr - Media lot clearer. ...?

12 hr TT - log computer @ 518. stopped saved experiment. restarted. → run to gas

12 hr TTc looks pretty good.

DTK - lot DES. more robust PPM?

12 hr - PPD running @ 700 cells/sec.

8:56 redlined sheet fluid [4/4 left]

18 hr - Media - fan-like IFNG PPD, a lot more  
TNFα producing cells. as you extend timeline.

9:15 pm → Second screen wait out completely.

Running blind

24 hr rest - TT2C DTC all got a tiny bit of sample when digest (undercounted/digested)

Done @ 10:56 pm



# Tube counts/Plate Counts/Supernatants

No Rest:	Projected	Actual
Thaw:		4:12pm
Count:		4:55pm
Plate:		
Process:		
Count:		
Stim:		5:29pm
Golgi:	7:29pm	7:30pm
Spin:	1:20pm	1:22pm
Spin2:		
L/D:		13:48pm → 2:03
Extra:		2:18pm → 2:38pm
Intra:		3:21pm → 4:09pm
End:		4:20pm

Media Costim TT TTA COS DTA COS PPD2 COS

w/ 10pl pipette antigens aliquoted

500pl

2:06 FBS-spendann  
Extra  
Intra @ 2:18 pm  
last FBS wash @ 2:37pm  
Perm wash → 2:50pm



6 hrs Rest:	Projected	Actual
Thaw:		8:26AM
Count:		9:06AM
Plate:		9:13AM
Process:	3:13 PM	3:10pm
Count:	3:45 PM?	15:38 pm → 3:52 pm brown
Stim:	4:00 PM	16:08pm
Golgi:	6:08 PM	6:09 PM
Spin:	12:00 PM	
Spin2:	12:20 PM	12:20
L/D:		12:30
Extra:		12:59
Intra:		14:24 pm
End:	3:04pm	3:16pm

Saturday, Jan 23rd, 2021

↓ lots of adherent M2s, but w/ cold wash only pried principals loose, not ideal. Error in how added warm media?

↓ PPD got less cells (420pl ~ 840pl?)  
w/ 10 pl pipette

Mass up tubes, think switched correctly

Media Costim TT TTA COS DTA COS PPD2 COS

840pl

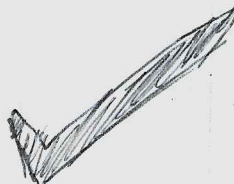
← Control  
← Not worth the time  
← Count better, strong sample, spin, wash, spin

th + LA mix @ 10:30 AM

Spin @ 13:22

✓ Fix Perm @ 1:40pm

2:06 Perm wash spin



2  
34 tubes/

\* 7

228 → 3 2/3 → 4 hours



Saturday

Saturday, Jan 23rd 2021

(R) (MCC) (T) (FEC) (DEC) (PCC)

175 → 350k cells  
↑ 500 pl, normal PPD etc  
but overcharged

1st FBS wash 6:47pm

Perm wash 7:31, 7:41pm 7:51 7:55 Last wash 8:03pm

Sunday

12 hrs Rest:	Projected	Actual
Thaw:		8:28 AM
Count:		9:06 AM
Plate:		9:13 AM
Process:	9:13 PM	9:03 PM
Count:	9:45 PM?	9:54 pm
Stim:	10:00 PM	10:14 pm
Golgi:	<del>10:14 PM</del>	<del>10:14 AM</del>
Spin:	6:04 pm	
Spin2:		
L/D:		6:30 pm
Extra:		6:58 pm → 7:18 pm
Intra:		8:11 → 8:51 pm
End:		9:08

← 7:18 pm



Saturday

Sunday

Sunday

18 hrs Rest:	Projected	Actual
Thaw:		4:12 pm
Count:		4:55 pm
Plate:		5:35 pm
Process:	11:35 am	
Count:		
Stim:		12:00 pm
Golgi:	2:00 pm	
Spin:	7:50 Am	
Spin2:		
L/D:		8:20 → 9:35
Extra:	8:55	8:55 → 9:15
Intra:		10:22 pm → 11:02
End:		11:27 pm

Need Warm RPMI & cold, + remnant Bio

Due to time conflicts bringing this forward 45 min, so ~13 hr-ish.

"Less adherent cells w/ longer culture."

Combined both tubes into one for this round.

Pre @ 15 ml, so ~13.46 E6/cells

10:58 AM spin down

11:16 SC stain Nodm MCC TT TTRC PPD DT alone

Spin Maria's Cent @ 8:40 am

DTx 1 DTx 2 DTx 3

DTx (~300pl) ~500pl ~600k

Spin Lid lidl issue → 9:24 pm → 9:32

9:36 golgi block, 9:47  
In the Biter Perm wash @ 10:00 AM

(R) (MCC)

10:36 (14/40) min







24 hrs Rest:	Projected	Actual
Thaw:		4:12pm
Count:		4:55pm
Plate:		5:35pm
Process:	5:35pm	5:03pm → into centrifuge
Count:		5:39pm
Stim:		5:53pm
Golgi:	7:53pm	7:49pm
Spin:	11:39pm	
Spin2:		13:57pm
L/D:		14:08pm → 2:23
Extra:	2:47	2:41 → 3:07
Intra:		4:03pm → 4:43pm
End:	<del>4:43pm</del>	

SCs @ 5:22pm

Media M+C TT TT+C DT+C  
 ○ ○ ○ ○ ○  
 ~ 820K

LD spin @ 2:26pm  
 → Issue w/ centrifuge rack

2:28pm

Find FBS with 3:07pm

3:20pm Perm Wash

8  
 x15  
 23

SCs / Fresh  
 Processed

12:41pm

12:59 resuspended  
 stain Count: Thaw 102

Count @ 1:22pm

1:29pm → aliquoted

1:31M extra cells → Trizol

2:17pm SC incubator 4°C

2:41pm

Spin @ 2:50pm

3:04pm done



"1 hour post thaw"

