

June 4 - 6<sup>th</sup>, 2021

Lena:  $\gamma\delta$  T cells: Expansion & D0 Staining  
David:  $\alpha\beta$  T Cells: 12 hr stim time course

12 hrs rest  
2+9, 12, 15, 18 hrs stim w/ golgi  
0.5  $\mu\text{g/ml}$  Costim ( $\alpha\text{CD28/CD49d}$ ) [2.5ul] &  $\mu\text{g/ml}$  TTx, DTx, PPD  
GolgiStop-Plug 1:1:19 (5)

Run on Tuesday 06/08/2021

@ 9:00 pm  $\rightarrow$  12:00 AM

$\sim$  11:45 pm  $\checkmark$

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly 1 <sup>100</sup>	Ly+Mon	Total	$\frac{1}{2}$	
ND050	Adult	-140°C	15E6	03/31/21	Lena yf's	3	2.89	4.34	8.67		
ND050	Adult	-140°C	15E6	03/31/21	David aB's	5	3.84	5.04	19.20		
ND050	Adult	-140°C	15E6	03/31/21	David aB's	1	3.62	4.22	3.62		
CVD4.282	Adult	-140°C	5E6		David aB's	4	4.41	6.06	17.64	8.82	4 = 4.320
ND050	Adult	-140°C	15E6			1	2.96	3.37	2.96	1.48	4 = 4.800

$\gamma\delta$ T cells Adult 1.5E+6 cells	Ex vivo Gd- $\alpha\beta$ PAb	Extracellular $\times 2.5$	Intracellular $\times 2.5$
V450 BV421	PD1	2.0	5.0
V525 BV510			
V670 BV650	CD56	1.8	4.5
B530 FITC	dq9		3 7.5
B710	CD3	1.5	3.75
PerCPeF710	Grnb		1.5 3.75
Y590 PE	NR22A	0.6	1.50
Y780 PE			
Vio770	CD3	1.5	3.75
R670 APC	CD3	1.5	3.75
R780 APC Fire	CD3	1.5	3.75
PBS	PBS	12.1	30.25

$\alpha\beta$ T cells Adult 1.0E+6 cells	Ex vivo	Extracellular $\times 20$	Intracellular $\times 20$
V450 BV421	CD4	1.8	0.5
V525 BV510	L/D Aqua	1:500	
V670 BV650	CD45RA	1.5	1.0
B530 FITC	CD69	1.0	1.0
B710	TNFA		3.0
PerCPeF710	CD62L	2	
Y590 PE	IFNg		0.8
Y780 PE Cy7	CD3	1.5	0.5
R670 APC	CD8	1.5	0.5
R780 APC-Fire750			
PBS		11.2	14.2

10.34 am CD45 count done.

12 hr (?) rest start @ 11:16 am

ND050 5/14ml

CVD 4282 1/14ml

same cell clone? present?

IL-2 out @ 2 weeks, keep 4%

12:30 resume

1:49 pm surface done  
2:09 pm FixPerm  
2:53 Indore  
 $\Rightarrow$  3.33

2.89  
 $\times 1.2$   
5.78  
28.90  
34.68

20:24 collected, spin down  
20:48 count stain  
9:06 count done  
32 costim

11:41 Inevitable  $\rightarrow$  11:41 pm  
 $\rightarrow$  golgi

Human IL-2 R&D @ -80°C  
Lot 9043 stock 200 ng/ml  
 $\alpha\beta$  15 ng/ml  
Infant 5 ng/ml  
Adult 10 ng/ml

10:12 & stim  
 $\rightarrow$  11:27

11:35 done pm

5ml volume 7.5E+6

1.5E+6/ml Lyemo

4.3E+6/ml  $\rightarrow$  1.5E+6/ml

1ml  $\rightarrow$  2.8ml

1.7ml = 7.5E+6 cells + 3ml R10

Culture 7.5E+6  
Stock 4.3E+6/ml (1.7ml)

vol x conc = cell #

28x

1.5E+6/ml

1.7 x 28  
= total  
= 47.6ml

Adult 5ml  
2d 1.100 + 50  $\mu\text{l}$

IL2 100 ng/ml (1:200) 25  $\mu\text{l}$   
#9043  
stock 200 ng/ml

9 hrs stim:	Projected	Actual
Thaw:		
Count:		
Plate:		
Process:		
Count:		
Stim:		
Golgi:		
Spin:	8:40am	
Spin2:		
L/D:		
Extra:	9:27am → 9:47am	
Intra:	10:47am → 11:27	
End:		11:47 ✓

☐ SEB  
☐ Media  
☐ TT  
☐ TT2c  
☐ costim

☐ PPD2c

20. Pippette head 16 ~~16~~

← reverse surface tips? (SEB avoided, others?)  
 10:47am → Cyt f12 / pen  
 ← draw from top vs bottom fix pen bottle?  
 last wash buffer rinse sat 6 min while waiting centrifuge.

resuspend in 250  $\mu$ l PFA-PBS

Done @ 11:47pm ✓

12 hrs stim:	Projected	Actual
Thaw:		
Count:		
Plate:		
Process:		
Count:		
Stim:		
Golgi:		
Spin:	11:25am ✓ @ 10:27	
Spin2:		
L/D:	11:39 → 11:54	
Extra:	12:09 → 12:29	
Intra:	13:30 → 14:10	
End:		2:21pm

☐ SEB  
☐ Media  
☐ TT2c  
☐ TT  
☐ costim

☐ PPD2c

Fix Penicillin 10 min  
 10 min @ 12:47pm

Done @ 2:21pm

15 hrs stim:	Projected	Actual
Thaw:		
Count:		
Plate:		
Process:		
Count:		
Stim:		
Golgi:		
Spin:	~2:25 pm = 2:26 pm	
Spin2:		
L/D:	2:40 pm → 2:55	
Extra:	15:10 pm → 15:30	
Intra:	16:24 pm → 5:04 pm	
End:		5:17 ✓

O SEB

O Media

O TF

O TR & C

O costim

OPPD & C

✓ last FBS wash  
was 12.5% FBS  
cell seed & done

6 Cyt/Arx @ 3:43 pm

Done @ 5:17 ✓  
pm

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

No L/D @ timepoints 9hr? (or all just dead? weird)



Conc @ 2m/ml so 500 $\mu$ l = 1M cells

$\star 3.85\mu$ l  $\sim 5\mu$ g  $\sim 10\mu$ g/ml  $\star$   
ind $\mu$ l

TTx  $\star 1.925\mu$ l  $\sim 2.5\mu$ g  $\sim 5\mu$ g/ml

DTx  $\sim 0.25\mu$ g stock  $\sim 2.5\mu$ g  $\sim 5\mu$ g/ml  
500 $\mu$ l  
 $\hookrightarrow$  stocky so mix w/ PBS

PPD 1mg/ml  $\rightarrow 1\mu$ g/ $\mu$ l  $\sim 2.5\mu$ l for  $\sim 5\mu$ g/ml  
500 $\mu$ l

SEB 1mg/ml  $\sim 50$  2.5 $\mu$ l  $\rightarrow 5\mu$ g/ml  
in 500

Q-32 cost

Costim  
5 + 5 + 42 = 2.5 $\mu$ l - 500 $\mu$ g

Golgi 1 + 1 + 19 = 20  $\rightarrow$  1 ml  
 $\times 7$  5  $\rightarrow$  500 $\mu$ l  
133 /  
18  
 $\times 5$   
90

15 M NDUSEO

	TTx	C	M	SEB	TT
9:	○	○	○	○	○
12:	○	○	○	○	○
15:	○	○	○	○	○

PPD a 12 15 (no control)  
○ ○ ○