

July 15-17<sup>th</sup>, 2021

#1

TTx/CRM197/PPD Optimization Experiment

Run on the LSR from 11AM → 4:18 pm

on July 17<sup>th</sup> → 2021 07 17 AB Antigen Costim Final (1.61 GB)

3300 µl  
4600 µl  
3600 µl  
300 µl

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+M	Total	1.0E+6	2E+6
ND050 a1-2	DT den.		15EG	03/31/21		6	3.73E6	5.43E6	22.4		
ND050 a3-4	DT den.		15EG	03/31/21		6	5.6E6	7.82E6	33.6	16.8	6=10.8
ND050 a1-4 (+/-)						6	3.95E6	5.83E6	23.7		
ND050 a5-6	DT den.		15EG	03/31/21		6	4.00E6	5.79E6	24.0		
ND050 a7	DT den.		15EG	03/24/21		3	3.58E6	5.28E6	10.7		
ND050 a5-7 (+/-)						7	3.92E6	5.11E6	27.44	13.72	7=6.72
ND060 (21172) Ault			15EG	06/3/21	Single colors	3	7.05E6	8.08E6		18.36	

3300 µl  
2300 µl  
800 µl  
6x6  
36  
521  
57

20.5 µl

Thaw start 6:56 AM  
Spin @ 7:16 am  
CD45.1 stain @ 7:44 am  
= 80.8 million cells ~ 11.5 lymphocytes

Cytokines	Ex vivo Aβ T cells	Surface µL x 60	Intra µL x 60
V450 BV421	CD4	1.8 108	0.5 30
V525 BV510	Aqua L/D	1:500	-
V670 BV650	CD45RA	1.5 90	-
B530 FITC	CD69	1.0 60	1.0 60
B710	TNFα	-	3.0 180.0
PerCPeF710			
Y590 PE	CD62L	2 120	
Y615 PE Dazzle			
Y780 PE Vio770	IFNγ	-	0.8 48
R670 APC	CD3	1.5 90	0.5 30
R780 APC	CD8	1.5 90	0.5 30
Fire750			
20.5 µl/rxn;	PBS	11.2 672	14.2 852

- Forget set cant gate @ 1000, 1200 spec.  
cant done @ 8:06 am

Going to do RT → RT media, let ↑ 37°C  
the incubator...

Incubator @ 8:30 → 61 M  
a1-4 : 46.1 million cells / 6 wells ~ 7.7 ml  
a5-7 : 34.7 million cells / 6 wells ~ 8.0 ml

Need to make more Rio, only have ~20 ml left.

6:21 pm Costim & antigen deliveries ready?  
Let us begin...

60  
21

Cytokines	Ex vivo Aβ T cells	Surface µL x	Intra µL x
V450 BV421	CD4	1.8	0.5
V525 BV510	Aqua L/D	1:500	-
V670 BV650	CD45RA	1.5	-
B530 FITC	CD69	1.0	1.0
B710	TNFα	-	3.0
PerCPeF710			
Y590 PE	CD62L	2	
Y615 PE Dazzle			
Y780 PE Vio770	IFNγ	-	0.8
R670 APC	CD3	1.5	0.5
R780 APC	CD8	1.5	0.5
Fire750			
20.5 µl/rxn;	PBS	11.2	14.2

Spin @ 6:57 pm - cold wash still low  
tappily tapped ✓

7:27 pm → cant incubation  
7:39 pm count

61 million cells, needed 57 ✓

8:06 plated Tacs

TT @ 8:17 RT @ 8:24 26.32

ink. Costims @ 8:45 RT @ 8:48

8:55 everything in

Doses → 16:37 ✓

Δcostim → Done @ 10:51 pm

96 → 48 ml  
48 → 24 ml

07/16/21  
Start @ 1:57 pm  
2:04 pm  
clamped @ 2:24  
100 @ 2:32 pm  
2:47 pm  
15:05 pm into  
cent  
30 @ 5:30 pm  
Spin @ 16:00 pm  
Fix Per @ 4:23 pm  
1st wash @ 4:51 pm  
5:36 pm → intra @ 4:48  
6:21 pm last spin  
Done before 7:10 pm

Blank page with three binder holes on the right side.

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	Projected	Actual
Thaw:		6:56
Count:		
Plate:		
Process:		
Count:		
Stim:		
Golgi:		
Spin:		
Spin2:		
L/D:		
Extra:		
Intra:		
End:		

	TT2C 1/1	TT	DT2C 1/1	DT	PPD2C 1/1	PPD	ND2C 1/1
20	○	○	○	○	○	○	○
10	○	○	○	○	○	○	○
5	○	○	○	○	○	○	○
2.5	○	○	○	○	○	○	○
1	○	○	○	○	○	○	○
Controls	cos	cos	Media	Media	SFB	SFB	

using these ↑ for these ↓

TT	NocD28	ID28 x1	CD28 x2
NocD49d	(+)	(1-)	(2-)
ID49d x1	(-1)	(1-)	(2-1)
CD49d x2	(-2)	(1-2)	(2-2)

And for both DT 2  
PPD as  
well

DT

○ ○ ○ ○ PPD

○ ○ ○ ○

○ ○ ○ ○

↑ a little shorter on cells

0.150  
x 60  
-----  
9.000  
900pl - 9 ml

2-10-1974  
1000  
100

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[Use part 2 screenshot the laser screen] /  $\rightarrow$  not recognized by FlowJo

R670 shot abit

B650 shifted gate moved peak back a bit

Probe a negative

Y590 G60 Y615 G40 L05 ✓

Academic's  
"experiments"  
"experiments"

V670 Focused me had to increase

11:41 am  $\rightarrow$  preliminary check staining looks good, ~~R670~~ Aqua looks weirdly shifted up, but otherwise normal?

TT 1-2 first full run... 4 minutes & change

10-15

4x57  
228

CD28 seems to help stretch...

3.5  
 $\times 30$   
105.0

2 hours  $\rightarrow$  4pm

1  $\mu$ l - 50 1:50  
0.5  $\mu$ l - 50 1:100  
0.1  $\mu$ l - 50 1:500?

Not costim driven but media/background

TT 2.5 @

Look also @ CD4 & CD8 - noise?

% T eff's / tube?

\* Low TT does look better o.o.o \*

more @ lower doses

CD3? CD4?

more smear...  $\downarrow$  in TT lower or not w/ DT high

Questioning my detection with a pipetting

18  
 $\times 4$   
72

Does TNF $\alpha$ /IFN $\gamma$  prod by CD3- a worry or not?  
what are the lymph CD3 gate doing?

TT done @

2:39 pm

DT done @

3:25 pm

Verify that it's not CD4 & CD8 high that are causing a non-specific issue...

High conc seem to do poorly...