

Sept 27-30, 2020

HEU: New TT Optimization #4

12 hr Rest, 2hr Stim, 18 hr Incubation.

Adult	Location	Date	Tasks	Volume	Ly	Ly+Mon	Total
JD050		5E6/ml 08/18/20	SEB 1-1, 1-500 TT 1-1, 1-500 (x, 2x 10 µg) Costim 1-1, 1-500 (x, 2x	4 ml	3.27 E4	4.30 E4	13.08 E6
				4 ml	2.72 E4	3.39 E4	10.88 E6

↑ 9 ml + 4

Adult 1.0E+6 cells	Ex vivo TT	Extracellular x 10		Intracellular x 10
V450 BV421	CD69	2	20	CD69 1.0 10
V525 BV510	Aqua	1:500	-	Aqua - -
V670 BV650	CD4	1.8	18	CD4 0.5 5
B530 FITC	CD8	1.5	15	CD8 0.5 5
B695 PerCPeF710	TNFA	-	-	TNFA 3.0 30
Y590 PE	CD62L	2	20	CD62L - -
Y780 PE Cy7	IFNg	-	-	IFNg 0.8 8
R670 APC	CD3	1.5	15	CD3 0.5 5
R780 APC-Fire750	CD45RA	1.5	15	CD45RA - -
PBS		9.7	97	14.7 147

Raise to 5.4 - 4 ↑

1.4 ml media

2N/ml

11- 3M for SC controls = 8 M cells

ND050

0 | 0 | SEB @ 1 condition
0 | 0 | TT @ 1 condition
0 | 0 | costim @ 3 conditions

500 ±
500 media

500 µl

SEB 10 µl = 5 µl

TT @ 10 µg = 3.57 µl

TT @ 5 µg = 1.786 µl

costim =

5 + 5 + 42 µl (pick 5 or 2.5 µl)

conc ND050: 170 cells/µl, clean start 7:35 pm

cells into media @ 7:45 pm

: 115 cells/µl → Nice pop of APCs though!

Cells into Incubator @ 10:19 am

Cells into Incubator @ 12:23 pm + 18 = 6:23 AM

Allergies/coffee morning: start time: 6:40 AM

Extracellular in @ 7:36 am

First Perm wash @ 9:07 am

Intracellular in @ 9:31 pm → 10:11

SEB 1-1
@ 1 µg/ml

SEB 1-500
@ 1 µg/ml adjusted

OTT
1-1
10 µg/ml
0.5 costim

OTT
1-500
10 µg/ml
1.0 costim
adjusted?

OTT
1-500
10 µg/ml adjusted
0.5 costim
adjusted

0 costim
1-1
0.5 µg/ml

0 costim
1-500
1.0 µg/ml
adjusted

0 costim
1-500
0.5 µg/ml
adjusted

~ 8 samples 1.1.19 = 10 µl

5.5.95 Gels Bloch

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