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
ND050		5E6/ml	08/18/20	SEB 1-1, 1-500 TT 1-1, 1-500 1x, 2x 10µg Costim 1-1, 1-500 1x, 2x	4 ml	3.27 E4	4.30 E4	13.08E6
					4 ml	2.72 E4	3.39 E4	10.88E6

↑9 ml+4

Adult 1.0E+6 cells	Ex vivo TT	Extracellular ×10			Intracellular ×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 ↑ 1.4ml media

2M/ml

11- 3M for SC controls = 8M cells

ND050

O	O	SEB @ 1 condition
O	O	O TT @ 1 condition
O	O	O costims @ 3 conditions

500 + 500 media ↑ 500µl ↑

SEB 10µl = 5µl

TT @ 10µg = 3.57µl

TT @ 5µg = 1.786µl

costim = 5+5+42µl (plate 50 2.5µl)

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

cells into incubator @ 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

Allergens/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 @ 1µg/ml ✓

SEB 1-500 @ 1µg/ml adjusted ✓

TT 1-1 10µg/ml 0.5 costim ✓

TT 1-500 20 10 5 µg/ml 1.0 costim ✓ adjusted?

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

O costim 1-1 0.5µg/ml ✓

O costim 1-500 1.0 µg/ml adjusted ✓

O costim 1-500 0.5µg/ml adjusted ✓

~8 samples 1·1·19 = 10µl × 5 = 5.5.95 GolgiBlock