December 18-21, 2020

(v18) HEU: DTX/CRM197 Optimization "#2"

Overnight rest, 2 hours stimulation, 18 hours incubation; +/- Costim, 5, 10, 20 ug doses.

1x or 2x Costim w/ reg Golgi Core ~ Million cells (vs volume) ml

Specimen	Status	Location		Date	Tasks	Volume	Ly	Ly+Mon	Total	Vol 3M/ml
200µl ND050	Adult TT/DT	-130°C	Rested	10/21/20		2.5	2.33	3.00 E4	5.75	2.875 - 2.5 = 375µl
250µl ND050	Adult IF	-130°C	Fresh	3ml	1.51E4/2.44	~1 ml (warm)	4.89	6.35E4		

* 4.53 million ‼ what? / 7.32 million L+M

Adult 1.0E+6 cells	Ex vivo	Extracellular * 3.5 (×2)			Intracellular * 3.5 (×2)	
V450 BV421	CD69	1	3.5	CD69	1	3.00
V525 BV510	Aqua	1:500		Aqua	-	
V670 BV650	CD4	1.8	6.3	CD4	0.5	1.75
B530 FITC	CD8	1.5	5.25	CD8	0.5	1.75
B710 PerCPeF710	TNFA	-		**TNFA**	3.0	10.5
Spin down → Y590 PE	**CD62L**	2	7.0	CD62L	-	
Y780 PE Cy7	IFNg	-		**IFNg** ×2	0.8	2.80
R670 APC	CD3	1.5	5.25	CD3	0.5	1.75
R780 APC-Fire750	**CD45RA**	1.5	5.25	CD45RA	-	
PBS		10.2	39.2		13.2	49.7

[highlight] running low antibody, waiting delivery

5ml - 10µl L/D Aqua
L/D @ 9:05 am
FBS spin 9:23 am
Surface @ 9:35am
Cytf/P @ 10:09am
Permwash @ 10:34 am
Intra @ 10:53am
Done @ 11: am

Thawed @ 5:35pm → 10ml media/2 wells.
Processing start @ 11:00 AM (17½ hours rest)
1st warm save FBS 11:11 AM (still good # of healthy cells)
Cold wait @ 11:18 → very few cells left, mostly adhered. Give 10 minutes on ice today.
In centrifuge @ 11:30 am (still the odd random cell no more than 3 per field of view)
Stain count @ 11:54 am
13 c/µl Ead → Virtually nada. 4 lymphocytes
Hot →
130 c/µl ND050 → [Thaw another for SC's!]

	Naive	TT	CRM197
+2x costim	48K/ML	1M	1M
+1x costim	4 1M	1M	1M

[Incubation start @ 12:49 pm] + 20h = ~8 AM

Let's use a scaled golgi block/plug approach this time?
1+1+19 ~ 10µl ~ 1M/1ml in 500µl? matter? vs 1M cells?

Let's stick w/ what works I guess... everyone gets 10µl for now, repeat w/ 5µl in January.

[2:49pm] Add Golgi Block.

13:02 Thaw new ND050 for SC's etc.
13:20 Inc Cell count
Bit more debris field on FSC x SSC.

4.53 M Total 1.51 M/ml
300K / 1.51M = 0.198

14:04 SC's inc start
2:20pm Leftover RNA in freezer
2:39 SC's done.

Cells in for 18 hr inc @ 14:49 pm so back by 8:49 AM

500 µl media ~ 1 million cells ~ $\frac{\text{Concentration}}{2M/ml}$

ΔGolgiConc

10 µg/TT/ml · million cells → 5 µg in 500 µl ~ 1.785 µl

10 µg/CRM197/ml · million cells → 5 µg in 500 µl ~ 0.5 µl of stock.

~~10 µg~~

Costim	Naive	TT	Crm197	PD
1x	○	○	○	○
2x	○	○	○	○

Costim 5 + 5 + 42 µl = 52 µl → 0.5 µg/ml

so 12.5 µl for a 500 µl volume

Golgi Plug/Golgi stop

1 + 1 + 19 µl = 10 µl for 1M in 1 ml? ← Previous focus # cells

5 µl for 1M in 500 µl

↑ ↓ Which factor matters most

3M single colors

5 µl - 50 µl 10 µl = 0.5 2x

1:10

5 µl = 0.25 × $\frac{250\mu l}{500\mu l}$ = 0.5 µg/ml effective 1x

18 ml 5% FBS - PBS

10%

1.8 ml

900 µl - into 17.1 µl through strainer.

10 ml 1 ml into 19 ml