Finished 2:34pm 2M/ml 3m = ~6million cells ~300,000/ 6.6 / 19.8 = 150.5 µl

20µl 5µl 1µl 1:100 500

Mar-09-2020

HEU: TT Stimulation
Overnight Intracellular Cytokine
Ex Vivo Staining

Adult	Location		Date	Tasks		Volume	Ly+Mo	Ly
CVD-280	10x6		11/9/19	Naive, TT, SEB		7ml	3.46E4	2.6E4
CVD-280			11/05/19	Naïve, TT, SEB				
				Naive				
				TT				
				SEB				
				Single Colors				

LSRII / lasers

Adult 1.0E+6 cells	Ex vivo TT	Extracellular	Intracellular
BV421 V450	CD69	2	
BV510 V525	Aqua	1:500	
BV650 V610	CD4	1.8	0.5
FITC B530	CD8	1.5	0.5
PerCPeF710	TNFA		3.0
PE Y590	CD62L	2	
PE Cy7 Y780	IFNg		0.8ul (2ul/rest)
APC R670	CD3	1.5	0.5ul
APC-Fire750	CD45RA	1.5	
PBS		9.8	15.7

Master Mix (x13 samples)	
26	—
—	—
23.4	6.5
19.5	6.5
—	39.0
26.0	—
—	10.4
19.5	6.5
19.5	—
126.1 µl	191.1 µl

20 µg/ml ?
~1300TT/RAMI
+700 RAMI volume to the TT

* costims only had 500µl volume @ 2hrs, added 500µl → uniform across group
so... 1µg/ml core vs .5µg/ml

Cell Prep: 4:30

Start Time: 6:07pm

Rag 11:50 - am 1:10 pm

Costim	Golgi block time:	Overnight Incubation	Spin down time
+2 hours	8:07	+10 hrs	6:07
+2 hours	8:07	+12 hrs	8:07
+4 hours	10:07	10 hrs	8:07
+4 hours	10:07	12 hrs	10:07

20µg/ml
·3
6µg ml + 20µg/ml = 26µg/ml
6.5µg/ml TT
20

LSRII, USE CST setting since Jul-11-2019

TAbs

BV 421 a-CD69 Biolegend
BV 650 @-CD4 Biolegend
FITC a-CD8 Biolegend
PerCP/Cy5.5 a-TNF-a Biolegend
PE @-CD62L Biolegend
PE-Vio770 a-IFNγ Mac Miltenyl Biotec
APC a-CD3 Biolegend
APC/Fire750 @-CD45RA Biolegend

Sbotr stains

BV 421
BV 510
BV 650
FITC
PerCp eFluor710
PE
Pe Cy7
APC
APC Fire 750

CD3