

Finished 2:34 PM 2M/ml 3m = ~6 million cells ~300,000/2.6 = 150.5 µl

20 µl
5 µl
1 µl
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	10x10G	11/9/19	Naive, TT, SEB	7m	3.4E4	2.6E4
CVD-280		11/05/19	Naive, TT, SEB			
			Naive			
			TT			
			SEB			
			Single Colors			

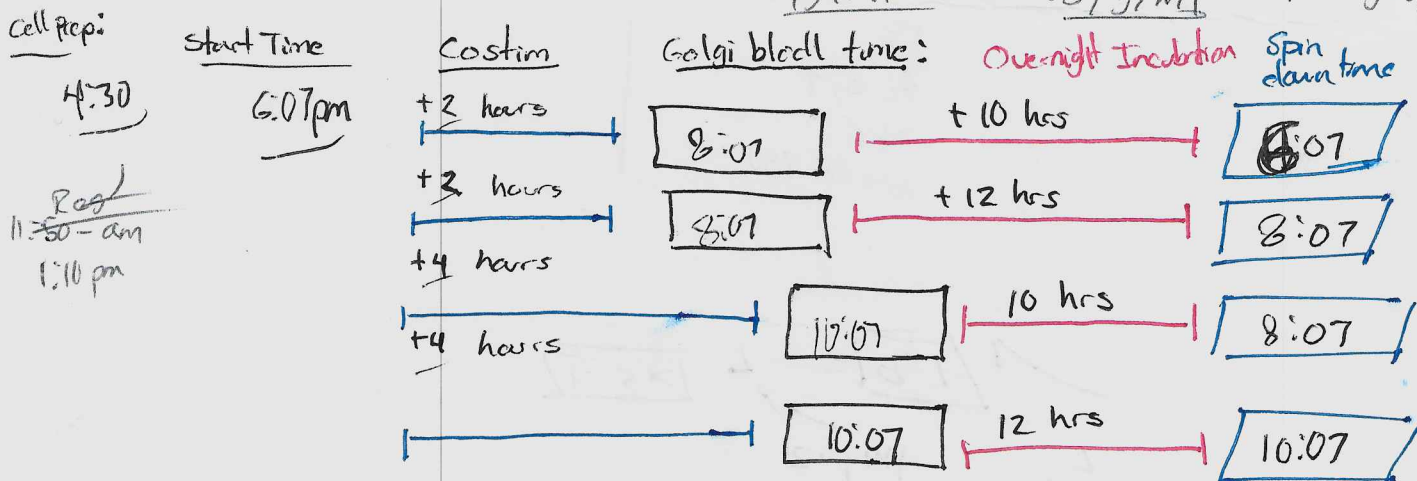
LSRII / ascs

Adult 1.0E+6 cells	Ex vivo TT	Ex vivo cells	Intracellular	Master Mix (x13 samples)	
✓ BV421 V450	CD69	2		2.6	-
BV510 V525	Aqua	1:500		-	-
✓ BV650 V610	CD4	1.8	0.5	23.4	6.5
FITC B590	CD8	1.5	0.5	19.5	6.5
PerCPeF710	TNFA		3.0	-	39.0
✓ PE Y590	CD62L	2		26.0	-
✓ PE Cy7 Y780	IFNg		0.8ul (2ul/test)	-	10.4
APC R610	CD3	1.5	0.5ul	19.5	6.5
APC-Fire750	CD45RA	1.5		19.5	-
PBS		9.7	14.7	126.1 µl	191.1 µl

20 µg/ml?
~1360 TT/RAM

+700 RAM
volume to
the TT

* costim only had 500 µl volume @ 2 hrs, added 500 µl uniform across group
so... 1 µg/ml conc vs 0.5 µg/ml



20 µg/ml

• 3

6 µg/ml + 20 µg/ml = 26 µg/ml

6.5 µg/ml
26 µg/ml

Table

BV 421 α -CD69 Biolegend
 BV 650 α -CD4 Biolegend
 FITC α -CD8 Biolegend
 PerCP/Cy5.5 α -TNF- α Biolegend
 PE α -CD2L Biolegend
 PE-Vio770 α -IFN γ Mass Miltenyi Biotec
 APC α -CD3 Biolegend
 APC/Fire750 α -CD45RA Biolegend

SSC stains

BV 421
 BV 510
 BV 650
 FITC
 PerCP eFluor710
 PE
 PE Cy7
 APC
 APC Fire 750

CD3

$$\sqrt{1.91} = 1.38$$

$$\frac{1}{2.6 \times 10^6} = 1.92 \times 10^{-7}$$

$$2.6 \times 10^6 / m$$

$$18.2 \text{ million cells} = 1.82 \times 10^7 \text{ cells} = 1.82 \times 10^7 / m$$

$$2.6 \times 10^6 / m = 1.92 \times 10^7$$

$$2.6 \times 10^6 = 1.92 \times 10^7 \times m$$

$$m = 1.35 \times 10^{-1}$$