Pw.190

Aug-5/6-2020

HEU TTx Stimulation #4
hr Rest + 4 hr Stim + 14/16 hour Acc
Ex-vivo Intracellular Cytokine Stain

Adult	Location		Date	Tasks		Volume	Ly+Mo	Ly	
CVD 4000 280		10E6	11.05.19	TT New, 4/14-16, Costim 1-2x, SEB		1 ml	1.23 E5	9.38 E4	0.669
ND006		20E6	07.08.19	SEB, Costim, SC		2 ml	1.16 E5	9.14 E4	1.179

Sc: ~~347~~ 1.2M

Sc = 87µl (500,000)

Adult 1.0E+6 cells	Ex vivo TT	Extra	Intra	Master Mix ×10	
V450 BV421	CD69	2	-	20	-
V525 BV510	Aqua	1:500	-	-	-
V670 BV650	CD4	1.8	0.5	18	5
B530 FITC	**CD8**	1.5	0.5	15	5
PerCPeF710	TNFA	-	3.0	-	30
Y590 PE	CD62L	2	-	20	
Y780 PE Cy7	IFNg	-	0.8ul (2ul/rest)	-	8 ×2
R670 APC	CD3	1.5	0.5ul	15	5
APC-Fire750	CD45RA	1.5	-	15	-
PBS		9.7	14.7	97.00	147.00

280 ~ 420 cells/µl (Guava)

ND006 ~ 680 cells/µl

6:10 pm Vortex RPMI gdg; stop ~ 500µl every well. cap Vortex, back incubator 8:10 AM

8:10 AM 4-14

Total 11:26 AM Intracellular

3:20:00 11:31 AM Done!

10:10 AM 4:16

13:17 done!

10g ~ 1 Liter

0.5 grams – 50 ml 1/20

Costim Mix

5 µl a-CD28
5 µl a-CD49d
42 µl RPMI

5 µl of basic mix = 0.5 µg/ml

Costim Mix

5 µl Costim Mix (x2) 10 µl
+95 µl RPMI (x2) 190 µl
200 µl (100 µl)

TT mix 1x

3.57 µl TT x 2 = 7.14
5.00 µl costim x 2 = 10.00
91.43 RPMI x 2 = 182.86
200 µl (100 µl)

TT mix 2x

3.57 µl TT = 7.14
+10.00 µl costim x 2 = 20.00
86.43 µl RPMI x 2 = 172.86
200 µl (100 µl)

SEB mix

2 µl SEB
+ 198 µl RPMI
100 µl 100 µl

Bref/Mon:

4900 RPMI x 9 = ~~4.5 ml~~ 4.410 ml
10 µl GolgiMix x 9 = 90 µl

Golgi Mix

3 µl Bref
3 µl Monensin A
114 µl RPMI

10 µl of Golgi Mix =

Everyone gets 500 µl

2:10 PM Costim + stims added
~~6:10 PM~~ for 500 µl + golgi stop.
8:10 AM, 10:10 AM.

Post-rest counts

$B_3 \rightarrow B_{10}$ TT_{1x} 4-14 TT_{2x} 4-14 TT_{1x} 4-16 TT_{2x} 4-16

SEB 4-14 Costim 4-14 SEB 4-16 ~~SE~~ Costim 4-16

20:5:1:500

$(5.62)(.347) = 1.95$ M cells
(400) $= 2.248$ M cells
$(5.75)(.347) = 2$
$(400) = 2.3$ M cells

3.55 E4 (4.05)	3.08 E4 (3.56)	2.90 E4 (3.42)	2.74 E4 (3.14)
4.45 E4 (5.01)	4.06 E4 (4.67)	4.37 E4 (4.88)	5.20 E4 (5.92)

Not good?

Missing some important meth. need buddy.