

Aug-13-2020

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HEU: TT Stimulation #5

Same Day Intracellular Cytokine Staining

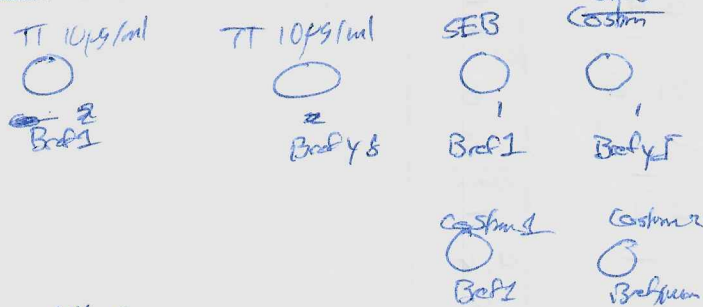
Ex Vivo

Adult	Location	Date	Tasks	Volume	Ly+Mo	Ly
ND050 1 10E4		11/5/19	TT SEB Costim 1x 2x Golgi Rest No Rest 2H, 4H	1ml	5.85E4	4.36E4
ND050 2 10E6		11/5/19	SC	1ml	7.54E4	5.96E4
ND006 5E6				1ml	3.74E4	3.15E4

4M/ml
6M/ml

Adult 1.0E+6 cells	Ex vivo TT		
V450 BV421	CD69	2	
V525 BV510	Aqua	1:500	
V670 BV650	CD4	1.8	0.5
B530 FITC	CD8	1.5	0.5
PerCPeF710	TNFA		3.0
Y590 PE	CD62L	2	
Y180 PE Cy7	IFNg		0.8ul (2ul/rest)
R670 APC	CD3	1.5	0.5ul
APC-Fire750	CD45RA	1.5	
		9.8	19.7

x12 for master mix
* Not enough for Rest Version



$$6 + 3.3 =$$

7:33-7:37 7:47-7:52 2nd batch
First batch: 1M/ml final \rightarrow 2M/ml \rightarrow plate 500 μ l \leftarrow add effector.

2-4 4-4 Always spin down
TT0

$$\frac{4.36 \text{ M/ml}}{2\text{M}} = 2.18 \text{ ml} - 1 = 1.18 \text{ ml for tube \#1}$$

$$\frac{5.96 \text{ M/ml}}{2\text{M}} = 2.98 \text{ ml} - 1 = 1.98 \text{ ml for tube \#2}$$

1m = .500
1/3 1/3
 $\frac{2\text{M/ml}}{300000} = 0.166 \mu\text{l per single color.}$
150 μ l

3.15 M cells / 1ml
1.575ml
- 1 = 575 μ l
 \uparrow 2M

Master Mix Extra x12	Intra
24	-
-	-
21.6	6
18	6
-	36.0
24	-
-	9.6
18	6
18	-
116.4	176.4