

~~Sept 21-23, 2020~~

HEU: New TT Optimization #6

0 or 12 hr Rest, +/- RBC Lyse, +/- CD69 Intracellular.

Adult	Location		Date	Tasks	Volume	Ly	Ly+Mon	Total
ND050			08/250		4 ml	265 E4	3.52 E4	10.60 Mln
					2 ml	2.45 E4	2.90 E4	4.90 Mln

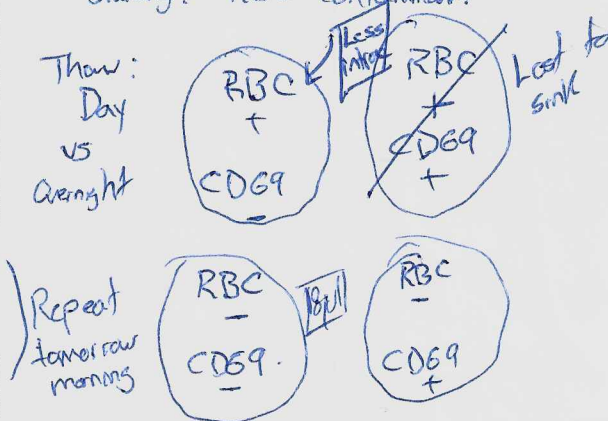
Adult 1.0E+6 cells	Ex vivo TT	Extracellular		Intracellular		
		\times	3		$\times 3$	
V450 BV421	CD69	2	6	CD69	0	0
V525 BV510 $\frac{3}{4}$ 20	Aqua	1:500	—	Aqua	-	—
V670 BV650 $\frac{7}{10}$	CD4	1.8	5.4	CD4	0.5	1.5
B530 FITC	CD8	1.5	4.5	CD8	0.5	1.5
B695 PerCPeF710 $\frac{2}{3}$ 4	TNFA	-	—	TNFA	3.0	9.0
Y590 PE $\frac{7}{10}$	CD62L	2	6	CD62L	-	—
Y780 PE Cy $\frac{4}{5}$ 5	IFNg	-	—	IFNg	0.8	2.4
R670 APC $\frac{7}{10}$	CD3	1.5	4.5	CD3	0.5	1.5
R780 APC-Fire750	CD45RA	1.5	4.5	CD45RA	-	—
PBS		9.7	29.1		14.7	44.1

Adult 1.0E+6 cells	Ex vivo TT	Extracellular x3		Intracellular x3	
V450 BV421	CD69	1.0	3	CD69	1.0 3.0
V525 BV510	Aqua	1:500	-	Aqua	- -
V670 BV650	CD4	1.8	5.4	CD4	0.5 1.5
B530 FITC	CD8	1.5	4.5	CD8	0.5 1.5
B695 PerCPeF710	TNFA	-	-	TNFA	3.0 9.0
Y590 PE	CD62L	2	6	CD62L	- -
Y780 PE Cy7	IFNg	-	-	IFNg	0.8 2.4
R670 APC	CD3	1.5	4.5	CD3	0.5 1.5
R780 APC-Fire750	CD45RA	1.5	4.5	CD45RA	- -
PBS		10.7	32.1		13.7 41.1

Questions about background.

Just messy donor? incubation?
staining? RBC contamination?

Thaw:
Day
vs
Overnight

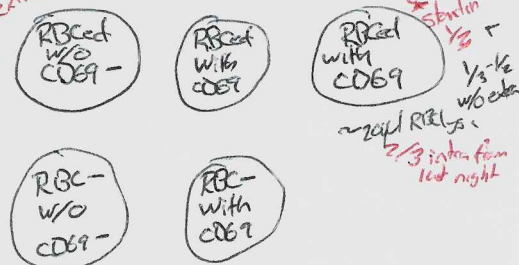


RBC + CD59 + went down
the drain \cap

10x RBC lysis conc instead
of 1x will do that

Day #2: Any extra go for tubes w/ RBC treatment...

17.5M extra
intra



Counting @ 5:45 pm
~200 cells/ μ l \leftarrow cells into overnight rest @ ~6:00 pm.

Intracellular start @ 8:13 pm

Last spin @ 8:55 pm

counting @ 100 um \leftarrow removed some cells
118 cells/pl so not quite equal

L/D 750 μ l 10:26 am \rightarrow 10:41 am

Surface @ 10:59 am

- 300 μ l RBC lysis

Are we going to lose any in the dbu? $\frac{11}{n}$? Yes/No

Intracellular in @ 12:20pm → 1:00pm

100