

September 23<sup>rd</sup>, 2021

HEU: hCD1d & a-Va24Ja18 dilutions #2

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+M	Total	1.0E+6	.3E+6
ND050 A	Adult		15E6	08/24/21		3	3.57E6	4.61E6	10.71	280 $\mu$ l	84 $\mu$ l
ND050 B	Adult		15E6	08/24/21		3	3.01E6	4.14E6	9.03	332 $\mu$ l	100 $\mu$ l

Tetramer Test iNKT	Ex vivo NKT-pla	Surface $\mu$ L	x 5.5
V450 BV421	Va24Ja18	2.2	—
V525 BV510	Aqua L/D	1:500	—
V670 BV650			
B530 FITC	CD4	1.8	9.9
B710	CD45RA	2	11.0
PerCPeF710			
Y590 PE	CD161	0.5	2.75
Y615 PE Dazzle			
Y780 PE Vio770	CD3	0.5	2.75
R670 APC			
R780	CD8	1.5	8.25
APCFire750			
20.5 $\mu$ l/rxn;	PBS	13.2	72.6

(19.5 aliquot) (100)

Tetramer Test hCD1d	Ex vivo FITC	Surface $\mu$ L	x 11
V450 BV421	CD4	1.8	9.9
V525 BV510	Aqua L/D	1:500	—
V670 BV650			
B530 FITC	Tetramer		
B710	CD45RA	2	11.0
PerCPeF710			
Y590 PE	CD161	0.5	2.75
Y615 PE Dazzle			
Y780 PE Vio770	CD3	0.5	2.75
R670 APC			
R780	CD8	1.5	8.25
APCFire750			
20.5 $\mu$ l/rxn;	PBS	13.2	72.6

loaded 2 1.5 1 0.5 5  
unloaded 0 0 0 0 0

Based on Cord Blood

R670 450 ~500 (450)  
 R780 450 (500) (500)  
 B530 450-500 (550) (450)  
 B710 450 (500) (450)  
 V450 450 (450) (400)  
 V525 500-550 (450) (not enough cells)  
 V670 450-500 (475)  
 Y590 550-600 (500)  
 Y615 600-650 (600)  
 Y780 ~500 (475)

50 unless we spike going fwd. + viable  
 75,000 ~ 1,200 Dead cells -  
 ~3000

Thaw @ 11 am  
 SE stain @ 11:25 am  
 1:46 pm spin down  
 2:01 pm L/O Aqua  
 Sds in @ 2:11 pm  
 PCVio770 @ 1  $\mu$ l (us-3)  
 (us PG7)  
 L/O Aqua @ 800  $\mu$ l  
 (instead 800)  
 for this round of experiments  
 2:33 & 2:38 tetramers  
 in  
 Sds done @ 2:44 pm  
 2:50 pm AMFI surface in  
 Invariant done @ 3:23 pm  
 Tets in surface @ 5:34 pm

reset FSC to 550

Warning - specified current > 9590 in 4615 is 126.43%

exp cyl settings  $\neq$  setup.  
all mine. in it

- Click apply to apply PWT voltage, threshold & comp values only for making parameters
- Click overwrite to replace all parameters & values w/ those from setup...

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OK, ignored the  $\square$ , hit apply, several times moving forward w/ it.  
- despite no appearance it did?  
- tubes have  $\checkmark$  PMT settings at least.  
V670 looks squished, @  $10^3 \frac{1}{2}$   
B530  $\checkmark$   
Y590  $\checkmark$   
-  $2\frac{1}{2}$  min

It's weird having everything anchored @ 0.0. -7 min  
- may need to FlowJo compensate this....

\* hCD1d  $\rightarrow$  I lose the crap signal @ 1:1500 range.

\* Solo 1:1500 contaminated w/ hCD1d @ equivalent amount solo.  
- competition assay?

Run done @ 9:50 pm

$\nwarrow$  adding on significant cleanup run time.

$1\frac{1}{2}$  for voltage walk  $\frac{1}{2}$  comp  $\sim$  2 hours for cells

Done @ 10:00 pm  $\checkmark$

$\frac{15}{\times 8} \overline{) 120} \sim 8 \text{ min/sample tube}$   
 $\sim 4 \text{ min/sample tube}$

