

Sept 6th, 24 PPD @ 9:08 am start

6022 @ 9:10 am

3/4/23  
15E6

NY066 15E4 3/15/23

@ 9:32 DNA=0

9:47 am

Cant done @ 10:01 am

$$C_1 V_1 = C_2 V_2$$

3239

3.2

3621

1.6 CUD  $\uparrow 3.8$  5  
1.8 NY068  $\uparrow 4.5$  6

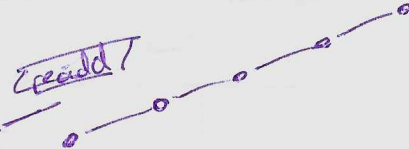
10:40 AM

10:31 am

Rest  
3M cells / 1 mL

remove 330 mL  
case

= 2M cells / mL



670  $\mu$ L for 2M cells

+ 330  $\mu$ L R10

+ 10  $\mu$ L PPD

NY068

750  $\mu$ L ~ 1.5

CUD 500  $\mu$ L ~ 1.0

30  $\mu$ L PPD

5  $\mu$ L

correcto 2M

35  
x 300  
10.500

15  
x 3.00  
4.5 million  
10.5  
15.0 million cells

16.3 + 18  
 $\mu$ g/mL 5  $\mu$ L / 10  $\mu$ L / mL

○ ○  
○ ○  
○ ○  
○ ○

○ ○  
○ ○

SEB  
○ 8.5  
○ 10.0  
○ 18.5  
○ 15.0  
○ 3.5

10  $\mu$ L

stine 3:47 pm  
9:54 pm  
2  $\mu$ L CUD A

5:58 pm stine  
9:41

Donor	Status	Collected ml	1:1 (x2)	# 50 ml /35	Connicals
ND006	Adult	34 tubes 490mls ~350ml	14		

Volume	Lym	Ly+M	Total

- Blood draw \_\_\_\_\_ mL (~10mL/heparin tube) from the donor.
- Dilute (1:1) blood with DPBS, bringing the final volume to be evenly divided by 35 mL
- Aliquot 15 mL LSM (or Ficoll Plaque Plus) in a 50 mL tube for required # tubes.
- Layer 35 mL of diluted blood on top of LSM.
- Spin 1500rpm, 27 min, Accelerate 6, Decelerate 2 [Option #1 Centrifuge] #14 50ml conicals
- Thaw freezing medium (Gemini A90F, cat 100-106); one tube of FBS, and one tube of FBS-20%DMSO(cat D2438, Sigma)
- Prepare 50 ml conical tubes and fill with 15 ml DPBS. Use transfer pipette to collect buffy coat into DPBS (combine two conical tubes into one). Fill up the volume with PBS as 50 mL.
- Spin 1000 rpm, 15 min, Accelerate 9, Decelerate 3. [Option #2] #7 50ml conicals  
\_\_\_\_\_ # of tubes = \_\_\_\_\_ tubes
- Estimate the pellet (~40E+6 in a tube). Dump the supernatant. Tap to dissolve the pellet. Add \_\_\_\_\_ mL DPBS in each tube and USE Transfer pipette to combine all in one tube. Add another \_\_\_\_\_ mL DPBS in each tube and collect cells again. Fill up with DPBS as 50 mL. Take 5 uL for cell counting (1:100) on the Guava.
- Spin 1200 rpm, 10 min, accelerate 9, decelerate 9 [Option #3]

Donor	Ly + Mono	Ly	Total Ly
100x dilution			
20x dilution			

Assume there are (250E+6) Ly for freezing  
Residual volume with cell pellet is 200 uL. Freeze 10E+6 cells in 1 mL, total \_\_\_\_\_ (25) vials.  
Resuspend cell pellet in the final volume of \_\_\_\_\_ (12.5) mL FBS ( ) while counting.  
Prepare \_\_\_\_\_ (12.5) mL FBS-20%DMSO  
Label (25) vials and chill on ice. Unscrew the caps.  
Dispense 500 uL of cells, then adding 500 uL of FBS-20%DMSO.  
Screw the cap and place vials in the freezing container.  
Flip several times and store at -80°C for overnight before storing at -140°C.

\*\*\* Calculate final volume: \_\_\_\_\_ mL blood \* 2 = \_\_\_\_\_  
Calculate number of 50 mL conical tubes needed: Final Volume \_\_\_\_\_ / 35 mL = \_\_\_\_\_ tubes  
Ex. If you end up with 6.2 tubes, aim to dilute as if you had 6.  
Number of tubes \_\_\_\_\_ x 35 = Final Volume = \_\_\_\_\_ - collected blood = \_\_\_\_\_ DPBS to add



αβ T cell SFC panel														
#	Filter	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	L/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	ColdStain 30min @4C	4°C for 15 min	RBC Lyse, then FixPerm	Intranuclear Stain 40 min @RT	8
1	UV2	BUV395	CD62L	(DREG-56)						1.2	9.6			8
2	UV7	BUV496	CD8	(RPA-18)						0.7	5.6			
3	UV9	BUV563	CD69	(FN50)										
4	UV10	BUV615	GCR4	(LG1)										
5	UV11	BUV661	Vα2	(B6)					2				1	8
6	UV14	BUV737	OXCR3											
7	UV16	BUV805	CD4	(SK3)										
8	V1	BUV421	CD127	(A019D5)						0.5	4		0.1	0.8
9	V3	Pacific Blue	CD14	(M5E2)					1.5	1.3	10.4		0.5	4
10	V5	Pacific Blue	CD19	(H1B19)										
11	V7	BUV480	CD161	(REA631)					1.5	12				
12	V13	BUV510	CD43RA	(H1T00)					1.5	12				
13	V14	BUV750	GCR6	(J252D4)					1.5	4				
14	V15	BUV786	GCR6	(3C10)					2.5	20				
15	B2	Alexa Fluor 488	FoxP3	(B27)					1.5	12			1.5	12
16	B3	Spark Blue 550	CD3	(SK7)										
17	B9	PerCP-Cy5.5	CD26	(M-A261)						1.2	9.6		3	24
18	YG1	PE	PD1	(EH12.2H7)									0.5	4
19	YG3	PE-Dazzle594	TNFr	(MAB11)						1.2	9.6		2	16
20	YG5	PE-Cy5	CD25	(M-A251)									0.1	0.8
21	YG9	cFluor-BYG750							1.2	9.6			1.2	9.6
22	YG10	PE-vio770	HLA-DR	(L243)									0.1	0.8
23	YG10	PE-Fire810	CCR7							0.8	6.4			
24	R1	APC	CD39	(A1)						1.0	<Add>		1	<Add>
25	R2	Alexa Fluor 647	IL2	(M01-										
26	R4	APC-R700	CD107a	(H4A3)									1.2	9.6
27	R6	Zombie NIR	L/D	(H4A3)										
28	R7	APC/Fire 750	CD27	(O323)										
29	R8	APC/Fire 810	CD38	(H1T2)						1.5	12			
And UNSTAINED CONTROLS !!!										1.5	12			

0.69 + 0.75 IL-2 ✓

0.51 + 0.91 ITNG / 10pl → 0.99 + 0.78 5pl ✓

1.11 1.92 5pl ✓  
0.87 1.52 ITNG 10pl ✓

