Sept 6th, 29 PPD (2 9:08 am Hart	
6022 C 9:10 am		
3/0/23		CIVI = CZ VZ
Ny1068 15£4 3/15/27,	72	79/ 3.2
@ 9:32 DAVA = C	(32	39/ 3.2
9:47 cm		
Court done @ 10:01an		6211
1.6 CUD M3.	5) 6	10:40 AM 10
10.31am (3M cells/1 ml;) =	1000 1111	eadd?
9	670 pl -	Scr 2M cells 30H RIO + 10H PPD
750 pl n 1.5 Swo Scopl 2 1.0	T30 pl ppdl	> coreto 2M
35 15 300 × 3.00 4.5 million 10.5 15.0 Million cells	16.3 + 18 Main 5/1/10/1/m SEB 0 0 0 0	1
3:47pm 3:54 pm 2 Kl C007 A		15.0
5:58 pm	< 9,41	

smob h

	C	Volume

# 50 ml Connicals	58/	1:1 (2x)	Collected ml	Status	Donor
	hl	1300E~ 1500E~	24 teles	JlubA	ND006

- 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 .2
- 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.
- 6. Thaw freezing medium (Gemini A90F, cat 100-106); one tube of FBS, and one tube of FBS-20%DMSO(cat Spin 1500rpm, 27 min, Accelerate 6, Decelerate 2 [Option #1 Centrifuge]# 14 Sont connect
- Prepare 50 ml conical tubes and fill with 15 ml DPBS. Use transfer pipette to collect buffy coat into DPBS D5438' 2igma)
- 8. Spin 1000 rpm, 15 min, Accelerate 9, Decelerate 3. [Option #2] The Solve corn, 001 (combine two conical tubes into one). Fill up the volume with PBS as 50 mL.

sedut = sedut to #

collect cells again. Fill up with DPBS as 50 mL. Take 5 uL for cell counting (1:100) on the Guava. each tube and <u>USE Transfer pipette</u>! to combine all in one tube. Add another ____ml DPBS in each tube and 9. Estimate the pellet (~40E+6 in a tube). Dump the supernatant. Tap to dissolve the pellet. Add _______ DPBS in

10. Spin 1200 rpm, 10 min, accelerate 9, decelerate 9 [Option #3]

Jm			
20x dilution			
ηш			
100x dilution			
	γ + Mono	гА	Total Ly

Flip coveral times and store at -80*C for overnight before storing at -140*C.
Screw the cap and place vials in the freezing container.
Dispense 500 uL of cells, then adding 500 uL of FBS-20%DMSO.
Label (25) vials and chill on ice. Unscrew the caps.
Prepare (12.51) mL FBS-20%DMSO
Resuspend cell pellet in the final volume of <u>(2.51) mL</u> FBS () while counting.
Residual volume with cell pellet is 200 uL. Freeze 10E+6 cells in 1 ml, total (25) vials.
Assume there are (250E+6) Ly for freezing

DPBS to add	collected blood =	- = 9muloV lani7 = 28 x	Number of tubes
, , , , , ,		5.2 tubes, aim to dilute as if you had	Ex. If you end up with (
səqnı ———	_ = Jm 25 / əmul	on ical tubes needed: Final Vo	Calculate number of 50
	, 207	= 7 * boold Jm	:9mulov lenif əteluəle ***

2010 e Islan 1951 Hypride/ was stun pref -77:05cm J.S.J. 0.734 の意思な 700 0 8:01 コンジが Golgi Block -Ś Out @ 5:49 am. 3F1+ 7.5 mls 2 ml NuclearWash 1500 rpm 6 min Second Perm Wash: 2 ml NuclearWash 1500 rpm 6 min Add Intracellular Stain, incubate @ RT for 40min First PermWash: 2 ml NuclearWash 1500 rpm 6 min Second Perm Wash: 2 ml NuclearWash 1500 rpm 6 min Lebra 2 gras 110 Ses sance cultur (vortex every 10 minutes) @1.8 am Wash with 1 ml PBS, spin down 1300rpm 8min 800 ul of LiveDead mix (1.2500) @RT for 15min Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min Add CD161 biotin antibody for 10 minutes at RT 1 ml Nuclear FixPerm, incubate @ 4C for 30min Add Streptavidin Mix, incubate @ 4C for 15min Wash 2 ml 5% PBS-FBS 1400 rpm, 6min Add HotStain mix, incubate @ 37C for 30min Wash 2 ml 5% PBS-FBS 1400 rpm, 6min Add ColdStain mix, incubate @ 4C for 30min Add 300-500 ul 1x RBC Lysis for 3 minutes Wash 2 ml 5% PBS-FBS 1400 rpm, 6min Cap tubes, wrap rack in foil, store at 4*C Resuspend in 30 ul 0.4% PFA-PBS 19/6 First PermWash: 18,76 24 4 4 16 0.8 9.6 0.8 97.6 9.6 Intranuclear Stain 40 min @RT αβ T cell SFC pane 12.2 24.4 33.6 10-40 Hzc 0.1 RBC Lyse, then Fix/Perm Strep 9.6 10.4 6.4 <Add> 12 12 124.8 249.6 12 9.6 9.6 V(,75,ms7) ColdStain 30min @4C 2-1,5 m/s/wash 1.3 1.5 0.5 1.2 1.5 15.6 31.2 43.8 1.2 0.8 1.5 mls 13.8mls 2.4 mls 12.9 **103.2** 25.8 **206.4** 35.7 16 12 12 20 12 9.6 9.6 82 m1 5x fos-PBs Hot Stain 1.5 12.8 23.2 mls Fix (02.0 wash 12ml +3 Pippette draw Antibody Total Brilliant Stain L/D RT 15 min N. 40.0 16m 9,00 54 m #### During stim!!! 20.5 3271.5 2003 × 6 == 32565 735= 8 + 0°0 × 2 32 7 0.40 Vial Lot # Pippette draw volume Antibody Total 0 * 0 R10 Media (REA631) Marker FoxP3 thesh O.4% PFA K27m 15 | 10 | V7 | BV510 | C | L | V10 | BV605 | C | V11 | BV650 | C | V11 | BV650 | C | V12 | BV760 | C | V13 | BV780 | C | V14 | BV780 | C | V14 | BV780 | C | V15 | BV10 | B And UNSTAINED CONTROLS !!!
 21
 VG9
 PE-vio770

 22
 YG10
 PF-fire810

 23
 R1
 APC

 24
 R2
 Alexa fluor 647

 25
 R4
 APC-R700

 26
 R6
 Zombie NIR
 Fluorochrome Pacific Blue APC/Fire 750 3UV561 300/ September 6th, 2024 Filter 1 UV2 3 UV9 4 UV10 5 UV11 6 UV14 6 UV16 7 V1 8 V3 9 \

0.69 + 0.75 ILZ 0.51 + 0.91 ITNG/10pl 60 0.99 + 0.78 5 pl 1.11 1.92 5 pl .67 152 Italy 10pl

test south;

Dow 12:22 pm your my os m 20:21 50/0/25 mg 22:11 1st perm @ 1057an @ 11:09 (4 the inhore Ist week) पटल प्रमा निकार पटिया निकार स्थापित स्थाप नाइप Sc mile @ 10:20 - 911:20 tou 4 may 6 10:12 an 1 shipt @ 9:55 -> 10:10 cm Sels fust beat Q 9:3 & 2nd wash & 10:09 lu/6 for bol @ giga 1 bz: b @ 254 x1

THE- 28 6 22 2- Uib 0 575 13 73

HASES & 2017/01/2 @ 8:80 AM

(08 SCB FBs web @ 8:34 pm