January 3rd, 2023 PPD, ele agbea? 1.5 E+5 Specimen Status Location Conc Date **Tasks** Volume Ly Ly+Mon Total 3E+6 .3E+6BC6+ 2x CVD4,282 10/12/20 donor 10E6 1.2 8.45 11.3 313.56 179mla Adult ? NY060 15E6 06/03/21 2 41173ml 7.03 8.65 skeptical enough 3004/1 CVD4.282 15 1.5E6 11 2 4.03 8.06 Leay 372H 3804/1 N40BO 2 13.6 6.80 8.85 01/03/23 Doing polystyrene plates as it is all that we have ... Using a 12 well instead of a 6 well (cells - closer) Count @ 8:50 pm 01/03/2023 No more than 4 mb/well next form CUD ~ 11.43 pl ~ 8.7 M - 2 wells NY060 ~ 13.5 Pl ~ 7.4 M > 3 wells (tight At) and applets, don't do Incubation start @ 9:11 pm this fill again Cells mostly happy, desert cover surface both 61/04/23 9:10 AM collection start, remove excess RIV into 50 ml command recoiled up 5 ml papette. soon add 2 ml Flot warm RANI. wast 5 mm, tap I use I all next time, splatters) 10:08 stanfount (CUD4282, NYOGO, INYOGZZZ)) CUD ~ 1200 pl ~ 10 EG 3 x dropped during stain, may have lost a droplet? NY080 2 10.8 pl Golg, Block ABS Monogks 5:5:95 ~ 500pl CVD ictal @ 7 cust NYUGO Incobation start @ 11:26 am + golgibbell Golgi Hox added @ 1:48pm + c0107a Oct @ 9:11 Pmv 79:45:00 THLA-DR SE (+ CDIC/ S= 0 Ourst O[MOEO] LAb mix prepped @ 4:04pm? C Not MIX Nepped @ 4:35pm> BUSIO? CO45RA? All mix world done by 5:25pm @ C0107a/2 CVD4282 O ZambeNIR / O Zombie NIR/ CD101A PPD SC From CVD 4282

Into spin @ 9:38 pm

2052 BURN LD@ 22:00 pm (+ 3pl fo whn) -> 5pin wy
Covered FBs's @ 22:20pm Mon/Ab Sch @ 22:12 - 1042pm/ Schark nashes RT coll bictor next @ 22:33 (Man holding w) >> 11:17 pm 3 min RBC Lyse @ 22:44 pr -> Spin @ \$0:55pm 0.47 V @ 11:09-19-29 11:17 pm Het's dere Hot spin @ 11:22pm into 2nd permunsh C11:42 Cold Absine before 11:42 pm 1 25? Post RR lyse spine 00:12 am Morocyles 0.4% Stephanden 4°C Done @ 00,26 an C00.24 7 39

2824 CD 1018 ESD SC GROW CAD #4881

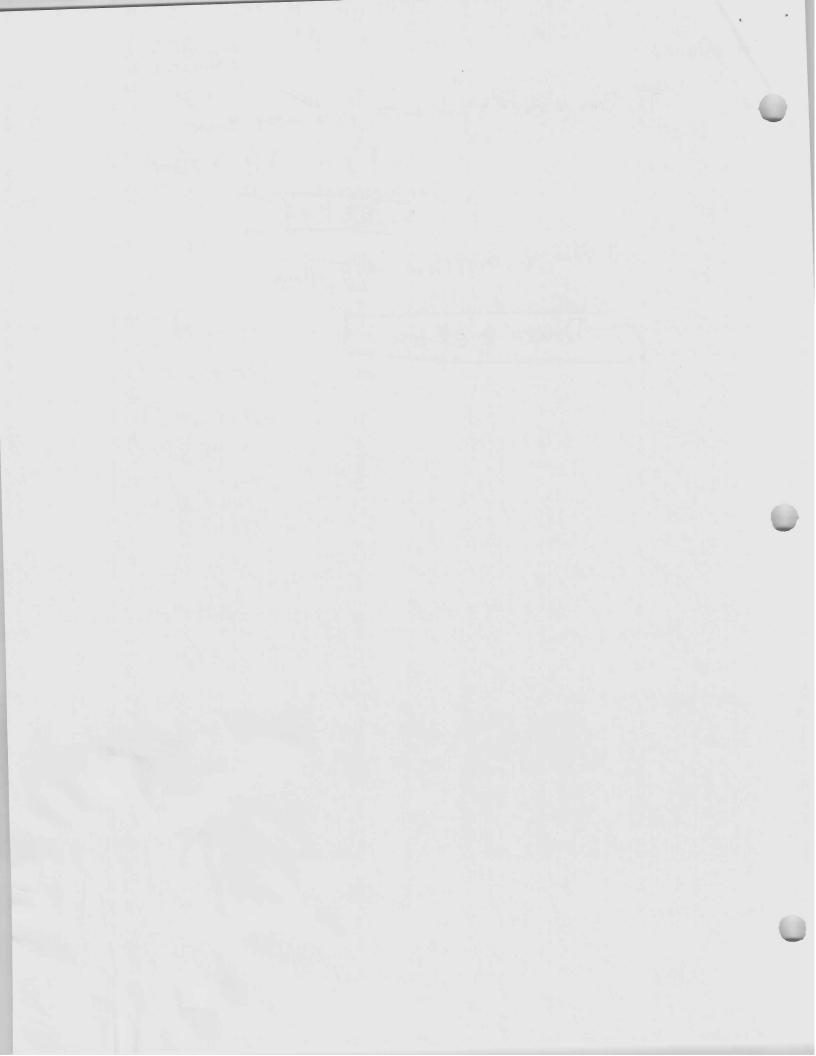
FIX Pean @ 00:53 pm -> 1:03 -> 1:15 am

2nd pen unt @ 1:23 am

555.2 pt

Intrac @ 01:31am - 2:11am

Done 2:22 am



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4 ColdStain 30min @4C		HotSta 30min @	blocker HotStain 3ullrxn 30min @37C	Vial lot#	Clone	Marker	Fluorochrome	Unmixing Ref ctrl type ctrl name		er (ul)	# Filter	

Simplified Protocol

January 4th, 2023

Thaw cells, DNAse, count.

Collect, count, aliquot cells 2.0E+6 Cells R10 / 5ml polystyrene tube Bring volume up to <500> ul R10, add <> Cap and incubate at 37°C for 6 hours

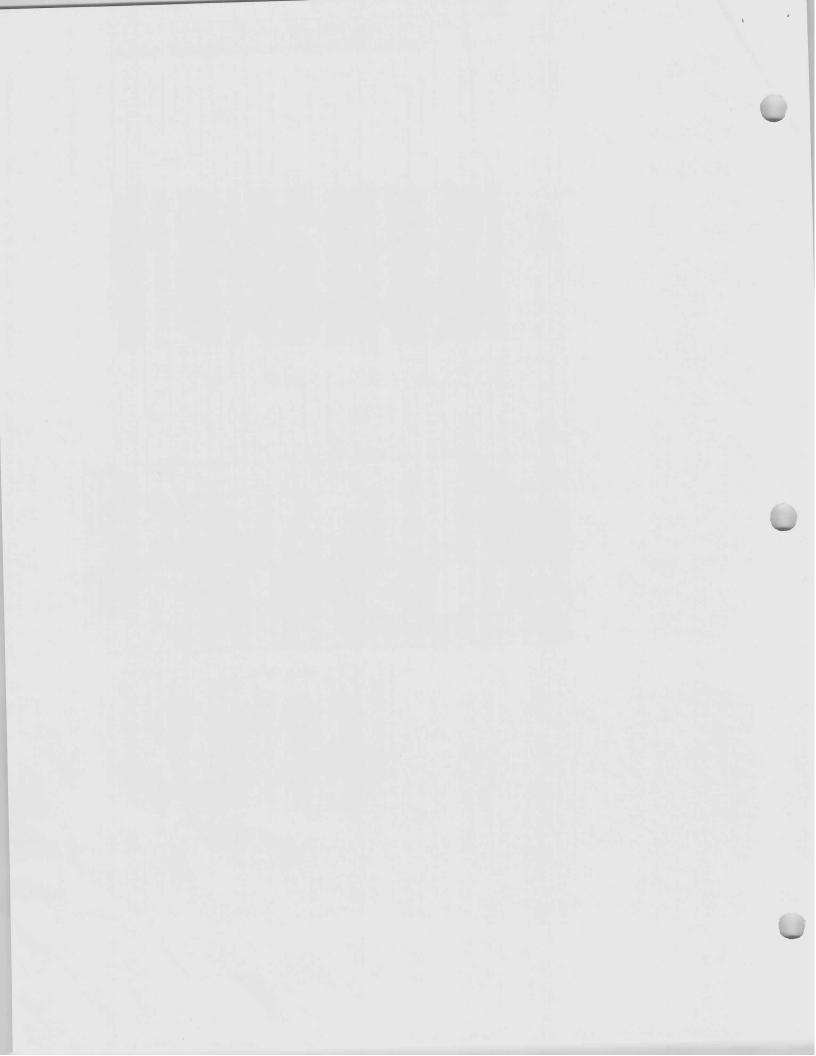
Wash with 2 ml PBS, spin down 1300rpm 8min 800 ul of LiveDead mix (1:2500) @RT for 15min Wash 2 ml 2% PBS-FBS + EDTA, spin 1300 rpm, 8min

Add tetramers for 40 minutes at RT Wash with 2 ml PBS, spin down 1300rpm 8min

Add HotStain mix, incubate @37C for 30 min Wash 2 ml 2% PBS-FBS + EDTA 1400 rpm, 6min

Add ColdStain mix, incubate @ 4C for 30min Add 300-500 ul 1x RBC Lysis for 3 minutes Wash 2 ml 2% PBS-FBS + EDTA 1400 rpm, 6min

Resuspend in 100 ul 0.4% PFA-PBS
Cap tubes, wrap rack in foil, store at 4*C



		And UNSTAINED CONTROLS !!!			29 R8	28 R7	27 R6	26 R4	25 R2	24 R1	23 B13	22 B10	21 B8	20 B6	19 B4	18	83	16 V15			1		12 V10		11 V7	10 V5	and the same of th	V3	9 V3	8 V1	7 UV16	6 U14	5 UV11	4 UV10	3 UV9	2 UV7		# Filter Single color (u
				APC/Fire 810	APC/FIRE /5U	20mble NIR	APC-R/00	Alexa Fluor 64/	Alcore	FE-VIO//U	BE WATE	ParCB-Cys s	BE CHE	DE-CERON	Spark blue 330	Spark him een	FITC	BV786	BV750	BV711	BV650	BV605	BV570	BV510	08440	BWASO	Pacific Blue	Pacific Blue	BV421	BUV805	BUV737	BUV661	BUV615	BUV563	BUV496	AF	BUV395	Unmxing ctrl Filter Single color (ul) Ref ctrl type name Fluorochrome
				CD38	CD27	L/D	CD107a	1112	CD39	HLA-DR	INFa	CDZS	CDZB	T-UA	CD3	TOAFS/ VUL	EUA EGAUS	CCRA	FNo	Va7.2	CCR7	CXCR5		CD45RA	Biotin	CD161 -	CD19	CD14	CD127	CD4	CXCR3	Vd2	CCR4	CD69	CD8	AF-UV6	CD62L	Marker
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Simplified Protocol

Thav cells, DNAss, court.

*Resulpend at 1Epril of 18 0, rest cells for <12>tes in a 6-well plate, >
Collect, count, aliquate cells 2 d6+6 Cells of n1 polystyrene tube
Add antigens, sima antibodies, and DuringStim antibodies in 500 ul R10
Cap and incubate at 37°C for 2 two hours
Add poligi block, and continue to incubate for <5> hours

Add CD161 biotin antibody for 10 minutes at RT Add to it HotStain mix, incubate @37C for 30 min Wash 2 ml 5% PBS-FBS 1400 rpm, 6min Wash with 2 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 ColdStain mix, incubate @ 4C for 30min Add 300-500 ui 1x RBC Lysis for 3 minutes Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

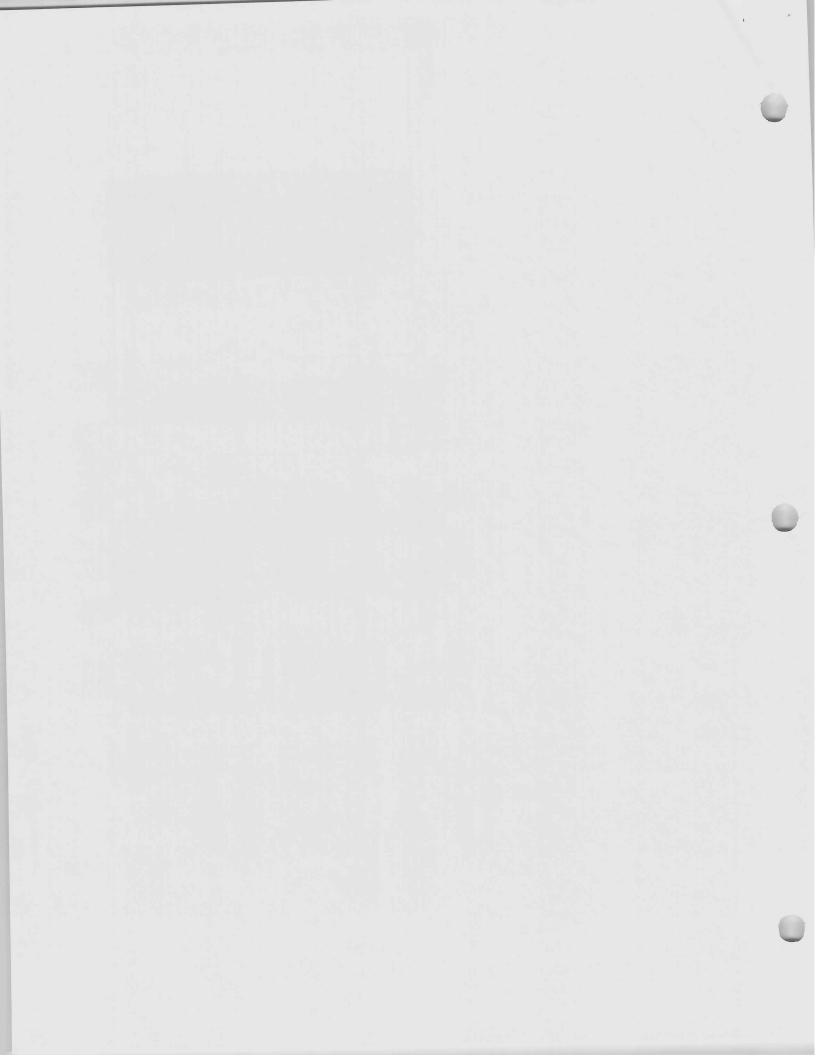
Add Streptavidin Mix, incubate @ 4C for 15min Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

1 ml Nuclear FixPerm, incubate @ 4C for 30min

First PermWash: Second Perm Wash: (vortex every 10 minutes)
2 ml NuclearWash 1500 rpm 6 min
2 ml NuclearWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min First PermWash: 2 ml Nuclear\
Second Perm Wash: 2 ml Nuclear\ 2 ml NuclearWash 1500 rpm 6 min 2 ml NuclearWash 1500 rpm 6 min

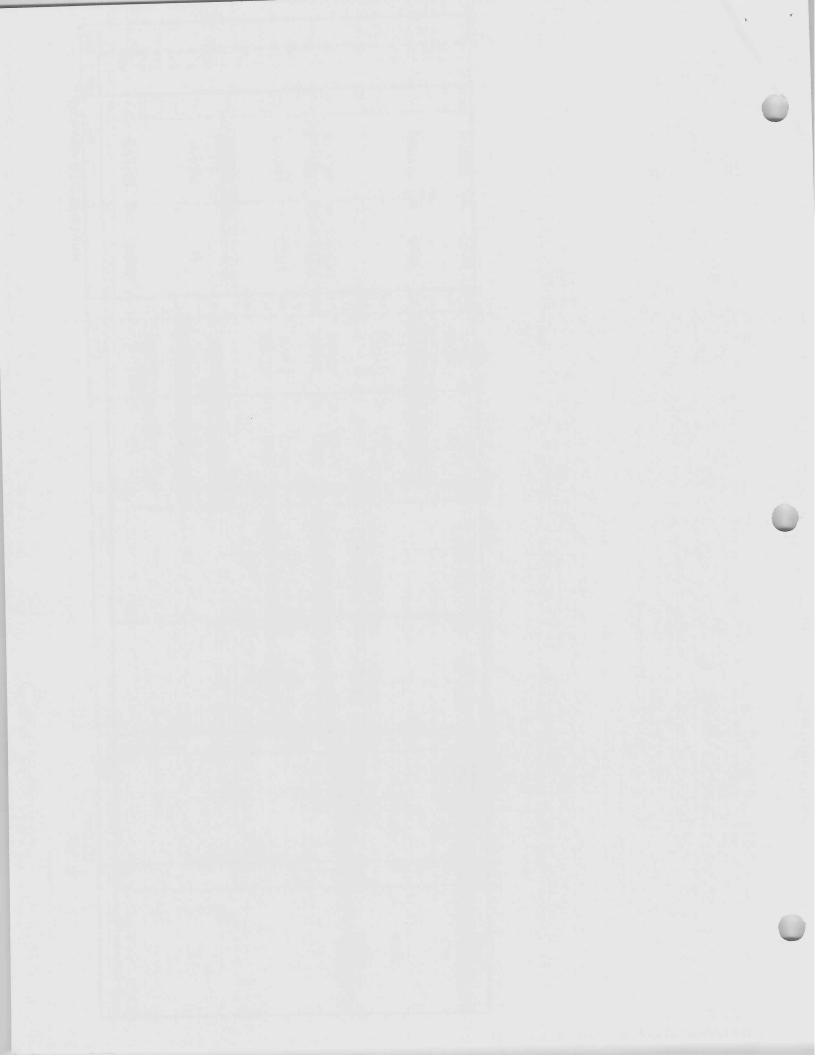
Cap tubes, wrap rack in foil, store at 4°C Resuspend in 100 ul 0.4% PFA-PBS



Monocyte & DC SFC Panel
Spectrum UV

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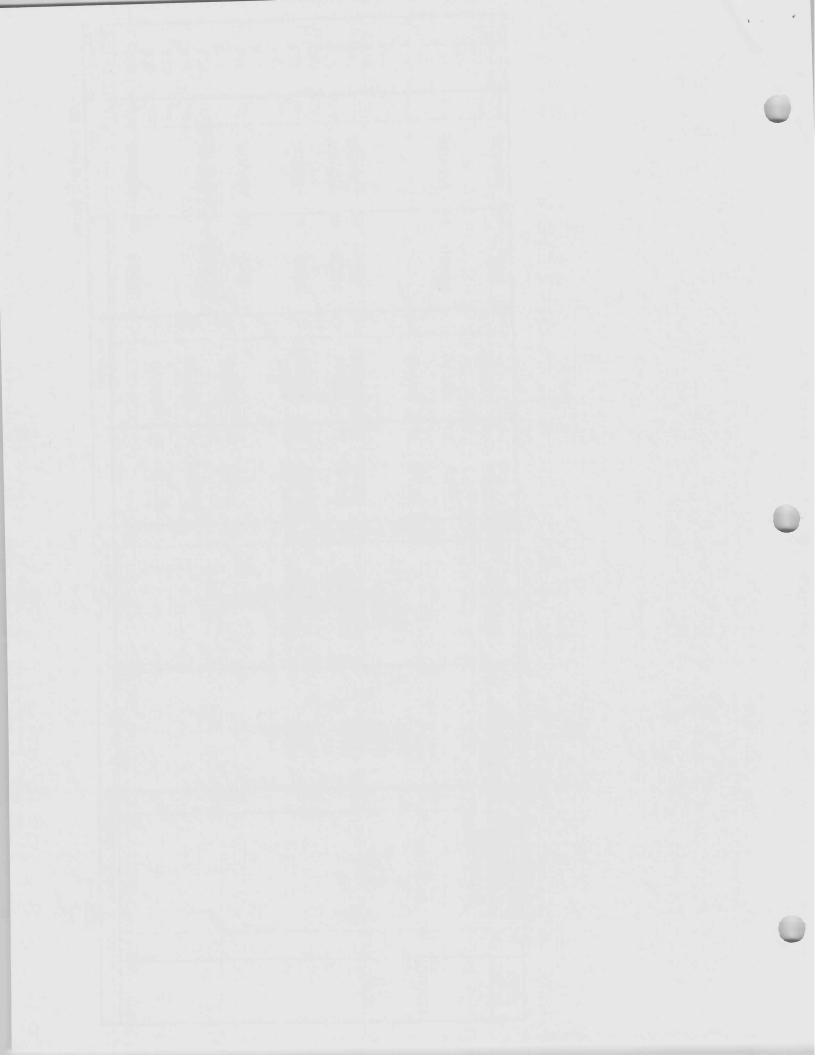
1/4/2023



AB T cell SFC Panel

Spectrum 388
428
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783 UV15 UV16 UV14 UV13 UV12 UV10 UV11 6AN 8AN UV7 970 UV4 UV5 UV3 UV2 LV1 V BUV615 BUV661 **BUV805 BUV737 BUV563 BUV496** AF-UV6 **BUV395** Ξ \Box [3] [2] [2] CXCR3 CCR4 Vd2 CD62L CD4 CD69 CD8 AF V15 V16 V14 V12 V13 V11 V10 **6** \$ \$ \$ \$ 5 5 Violet **BV786 BV750 BV711** BV605 BV650 **BV510** BV570 **PacBlue BV421 BV480** [2.5] [3] [3.5] $\overline{\omega}$ 4 [1.5] Ξ [4] 3 CXCR5 CD45RA CCR6 CD14/19 CD161 Va7.2 CD127 IFNg B13 B10 B11 B9 B8 В7 B6 B5 ВЗ В4 В1 B2 SparkBlue 550 PE PerCP-Cy5.5 Pe-Vio770 PE-CF594 PE-Cy5 FITC [4.5] [2] Ξ [1.5] [4] <u>7</u> HLA-DR CD25 TNFa CD26 Vd1 CD3 PD1 R7 R3 R2 R1 R₄ Red Zombie NIR APC-Fire 750 APC-Fire 810 APC-R700 APC AF647 [3.5] [3.5] 3 CD107a L/D CD27 CD38 CD39 IL-2

1/4/2023



January 3rd, 2023

Aurora on 8:54 am

PPD, ele agbea?

Last var removed the water tube? needle exposed.

Liz? or did I table tube off and larget? pheard it retract

