Specimen	Status	Location	Conc	Date	Notes	Volume	T	T .75	
CVD6022	Adult 37 806 Vex			03/04/23		2 ml	Lym	Lym+Mon	Total
	P			N	2 5 8 2 - 1	N 80131		Fiable	
						padid	ŧ₁ €	4	- AL

7:48 am thaw

7:55 Spin

LSL Aurora now on?

8:06 DNAse (25 pl)

8:16 Stainfor cant CIPIPE COHS; I PICDIA APC.

8:50 am Dilution estimates dene 8:55 am Cells into 372 Rest

IN coia pacBlue)

72:55 pm (for 6 hars)

@ 3MmL in ImL (4.7 mL's total)

New Golgi Block & stop arrived?

3:40 pm Cells out inaubator

4:03pm cells stand for recount

5:24 pm Cells into Incubeter

in antigen.

7:32 pm Golgi Block Added

-> 7:30 AM Start

Prepared done @ 8:05 pm/

6:46 and Nuclear washes make

7:31 am

7:41 stan for count ->

Cels spin down@ 1:47 am

Stashed 7.53

According St Cells almost 29mo 2:12

8:17 countstort 80 pl/min

8:26 count done sels aliqueting, blobuarteerd.

1-/0 @ 8:36 AM for samples → 8:

Hotse's @ 8:48 -> 9:18AM ->

Samples Hod Stein > 9:08 3 Cold Single Color > 9:11 -741 am

0 0 0 0 0 3 3 m/me in 1 m 1 5 H SER (Implan)

6 mL's need golgi black/step.

1:1:19 (10 pl / mL)

3: 3:57 | for golgir bref/marisin/pbs

2001 celb (se w/ 29 + 14 enit

get counts in the AM?

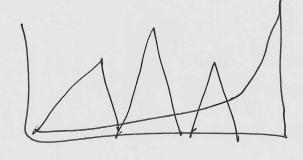
IL-21 / 6 in preview us

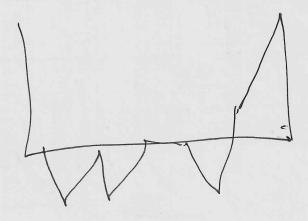
Hatzsis Carata Schot stashed w/vnstaneds Cold +5 amples spin @ 9:44 AM. cold of air samples @ 9:59 -> po:20 am / Sos Notar Fix @ 10:05 AM -> 15-> 25-> 35 am/ Cold wash samples @ 1030 AM Samples Strape 10:41 -> 10:56 cm (4c) Sels First nuclear worke Gening Second vash @ 16:58 AM + Streptudin striples @ 1500 vpm for 6mm (3:36 -> 11:04 / 31/2 has surface) 1 2 hrs mbracellary Sample nucleur fix -> 11:13 -> 23 -> 33 -> 11:13 Sc . htrastin 11:17am > 11:57am Se manh set done @ 11:26 w/ 30x1 # Samples Nuclear wash #12011:45
wash# 20 Sorples intracellar co 12:06 pm -> 12:46pm (se's intree # sod wish / No extra volume for intrancelear sybolines due Samples out @ 12:47 to arrival cell shipment Sar #3 samples @1250 pm #4@12:59 pm 1:12pm End

= 85 pl/min 3000 eurts SFC PPD Proof-of-Life 6:09pm High 106 0

1.5% CD4+/2.19% 9.14% CD4+ 38% CT8+

APC-Fic810





10.35

					Alid		1	26	25		24	23	22	20	19	18	17	15	14	13	12	 -	1	10	9		8	7	6	0	4 1	. ω	2	*
					NOIAIN		Z	3 R7	R6			R1 6	VG10	S S	YG3	YG1	B10		V15	V14	V13	<11	50	5	S	\(\sigma	3	<1	UV16	- 2	UV10	6AN	UV7	Filter UV2
					AIR ONS AINED CONTROLS !!!		APC/Fire 810	APC/Fire 750	Zombie NIR	APC-R700	Alexa Fluor 647	APC	PE-vio770	PE-Cy5	PE-Dazzie594	PE	Spark Blue 550	Alexa Fluor 488	BV786	BV750	BV711	BV605	BV570	BV510	BV480	Pacific Blue	Pacific Blue		BUV805	BUV661				Fluorochrome
							CD38	CD27	5	CD107a	5	CD45	HLA-DR	CD25	TNFa	PD1	65	FoxP3	CCR6	IFNγ	Va7.2	CXCR5		CD45RA	CD161	CD19	CD14	CD127	2	V52	CCR4	CD69	CDS	Marker
				Pippette draw volume /sample	K10 Media	Antibody Total	(HIT2)	(O323)		(H4A3)	(MIZ)	(24)	(L243)	(M-A251)	(MAB11)	(EITIZ.ZII)	(SK7)		(11A9)	(B27)	(3010)	(J252D4)		(Hi100)	(REA631)	(HIB19)	(M5E2)	(A019D5)	(SV2)	(B6)	(1G1)	(FN50)	(BBA-T8)	Clone
	Streptavi PBS	0.8		19.5	20.5	0.0		†	-	<r></r>	1	-		-+	†	†			+	+	-	H	1	†			+	+	-	-	+	+	 -	stimili
	3.2 78.8	1.6 4 5						1	unnunun	#####	Ī	-		7					1	1				+				-			+	+	-	ill 3
	w je			Pippette draw volume /sample	Brilliant Stain	Antibody Total		<0.22.1>	_	E																								15 min
		5 PBS 2 Zombie		draw Imple	5	otal																			<u>,</u>									RT for 10 min
				45.3	32.2	16.1	2 -	4			1.2		3.4	3				7.5	1			2.5					1.5	1.5		2.3	o n			30min @37C
PBS	Number	Number Number			96.6	48.3	4.8				3.6		0.0	3.6				4.5			2	7.5					4.5	4.5	1	1.0	7,			· · · · · · · · · · · · · · · · · · ·
24.75 24.75	umber Intracellula Total	Number Samples Number Unstained Number Surface Scr		40.5	29	14.5				The state of the s		Ç,	200		1.2		13			1.2			0.5		2.0	2.0	1	4	26	0.7	0.5	0.7	1.2	ColdStain 30min @4C
	6 6				87	43.5						7:	3		3.6	9.0	30			3.6			1.5		0	8			2.1		1.5	2.1	3.6	ے د
Perm Water	4.5 49.5 44.55	Ш	40						/		-													<0.8>	Strep	ST	1		T I					4°C for 15 min
8.4 76	1.5	RBC Lyse Fix 0.9 0.6	1 1920																															RBC Lyse, then Fix/Perm
	14 21			38.7	27.8	13.9				1.2			0.5	1.2	0.5	2.5	4		1.5								0.0	0,5	0.5		-3			Intracellular Stain 40 min @RT
Fix 5.25 Diluer 15.75	42 0.84 18 0.18 84 1.17 75.6	Perm PFA 18 0.09 6 0.06	L		83.4	44 7				3.6			1.5	3.6		7.5	12		4.5									2	1.5		ω			ω

αβ T cell SFC panel

≀ul of LiveDead mix (1:2500) @RT for 15min sh 1.5 (0.75) ml 5% PBS-FBS, spin 1300 rpm, 8min

I CD161 biotin antibody for 10 minutes at RT I HotStain mix, incubate @ 37C for 30min sh 1.5 (0.75) ml 5% PBS-FBS 1400 rpm, 6min

ColdStain mix, incubate @ 4C for 30min

sh 1.5 (0.75) ml 5% PBS-FBS 1400 rpm, 6min

Streptavidin Mix, incubate @ 4C for 15min sh 1.5 (0.75) ml 5% PBS-FBS 1400 rpm, 6min

(500) Nuclear FixPerm, incubate @ 4C for 30min (vortex every 10 minutes)

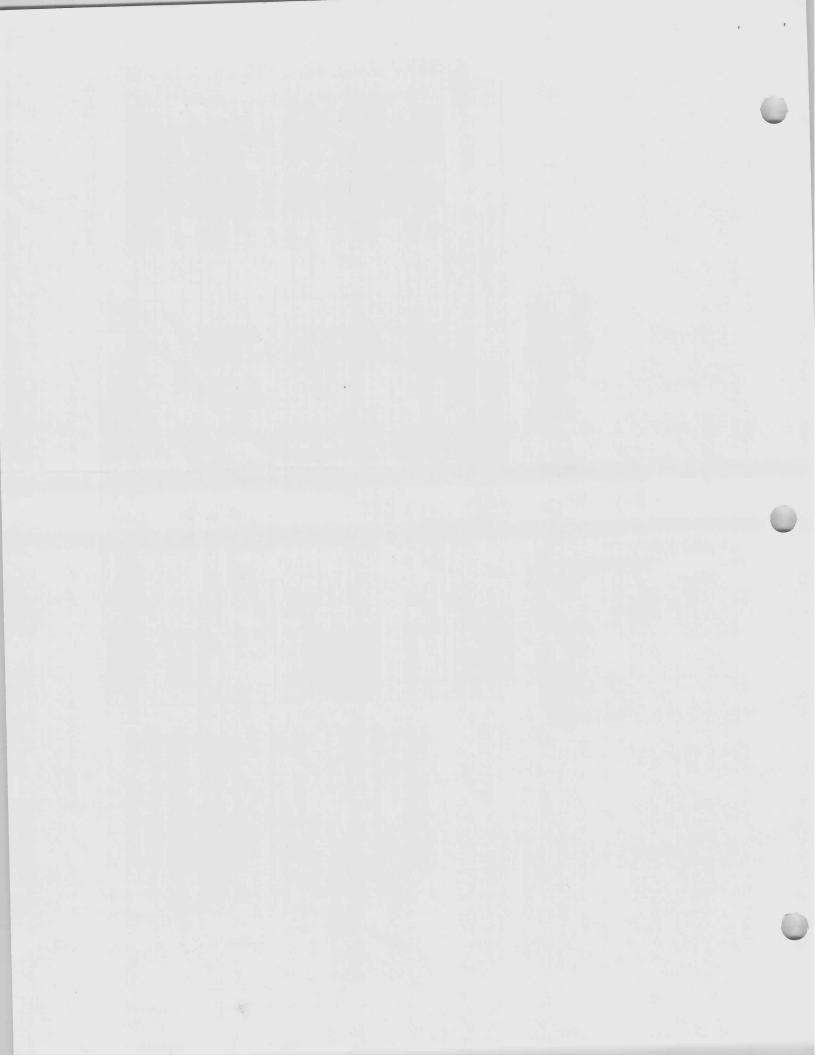
PermWash:

1.5 (0.75) ml NuclearWash 1500 rpm 6 min

and Perm Wash: 1.5 (0.75) ml NuclearWash 1500 rpm 6 min

Intracellular Stain, incubate @ RT for 40min PermWash: 1.5 (0.75) ml NuclearWash 1500 rpm 6 min vnd Perm Wash: 1.5 (0.75) ml NuclearWash 1500 rpm 6 min

spend in 50 µl 0.4% PFA-PBS ubes, wrap rack in foil, store at 4*C



783 uv15 812 uv16	750 UV14	697 UV12 717 UV13	664 uv11		542 UV8	508 514 _{UV7} 525	458 UV5 473 UV6	428 UV3 443 UV4	3/3 UV1 388 UV2	固
BUV805	BUV737		BUV615			BUV496			BUV395	VV
in Co	160		[50]	Ž.		(O) (A)			150	П
CD4			V82	69(1)		CD8			CD62L	
V15 V16	V14	V12 V13	V10 V11	§ §		V5		<u> </u>		<
BV786	BV750	BV711	BV605 BV650	BV5/0	BV510	BV480	PacBlue	BV421		Violet
[50]	[6e]	(55)	50 50		[36]	135	[55]	44		
CCR6		Va7.2	CXCR5		CD45RA	CD161	CD14/19	CD127		
B12 B13 B14	B11	B8 B10	B6 B7	B4 B5	83 B2 CO D	B1				œ
		PerCP-Cy5.5			Alexa Fluor 488 SparkBlue 550					Blue
		[45]			[56]					
		CD26			FoxP3 CD3					
469 4		YG5	YG3	YG1		-				1
Pe-Vio770 PE-Fire810		PE-Cy5	PE-Dazzle594	PE					rellow-Green	
50		Ē	[60]	[50]				*****	\forall	
HLA-DR		CD25	TNFα	PD1						
7 R R	R5 R4	73 R2	2			*			1	
Zombie NIR [55] APC-Fire 750 [45]	APC-R700	47	ABC	24					Red	July 9th, 2024
[55] Viability [45] CD27	CD107a	IL-2								

