	June <b>6</b> <sup>th</sup> , 20	23						2022		
- ^	Specimen	Status	Location	Conc	Date	Notes	V-1		ILT_06	
510c.	Inf694-9	HU 8	BOX ZOA CZ	18 ×17		110103	Volume	Lym	Lym+Mon	Total
10 C.	a-5	TNL2	(3)	(~?)			Inl	9.86	13.3	
ON		TNL3	1	(797			1	10.50	14.4	
oc.	Inf282-2 a-4	HEU-le SLP9	Box 8A F5	29.57		1 2 1	1	7.68	15.2	
30 C.		SLP8	9	9.47			1	8.00	13.9	
06.	Inf438-5 a-1	HEU-hi TURO	Box 13A DZ	(MIO)		enuran ural lead delvery		12.70	17.3	
P	a-3	TOR2		(7.67			1	11.40	15.7	
1	Inf 293-4 a=	SMAN	Ba 8A c7.	(25)		40,000 viral lead mon	1	5,15	7.32	
il -	G-D	SMAL	(P) <	20.57			1	5.08	7.65	
e.	ND050		9	15E6 < 11.77	1/13/23		l	9.17	12.0	
1	NDOOG			7.127	5/12/23	_	1	14.80	17.1-	
7:04 7:54 8:2	cord than adult than to cord than star 26 an com	2 0 m	6.57 7:18 m	C7:	3 an	1011 1505	· ·	000		Rallw ger 694, 2 438 ichae

[Incubate @ 9:41 am -> 3:41,07

zanhie kacas us canbie NIA

> only leftor" Of orhodillad

Als prepped between 1:10-1:40 pm

3:54 pm spindown samples 4110 pm 40 = 2= Spinch30 4:14 pm Sc spm/ost@4:26

4:31 pm Sc 40 -> 5:46pm 1 Roun

+ Sels = ~4:38 R= 4:43pm - 15:08pm Hot samples @ 4:50 -7 5:20 pm spin @ 5:20 pm Cold scise 5:00 -> 5.30pm

Tetse 5:42 pm -> 6:22pm-Abs @ 5:52 pm

50's FRE 5:49-75979

Leambined spin C 6.29 pm 2nd wash

Cold samples @ 6:47pm > 7:17pm 50 inter 6:52 -> 7:32 pm/

-1 6 4% PF1 e ziel pa

Rbc lyse samples @ 7:21 pm Spin 7:27 pm &

FIXP200 @7:41 -> 51-> 8:01 7.46pm so's done

1st wash @ 8.06pm 2nd washer 8:18pm

8:31 pm Intracellular - 9:11 pm 9: Bpn (Final Spin)

Done @ 9:25 pm

Interver 27

Interver 21

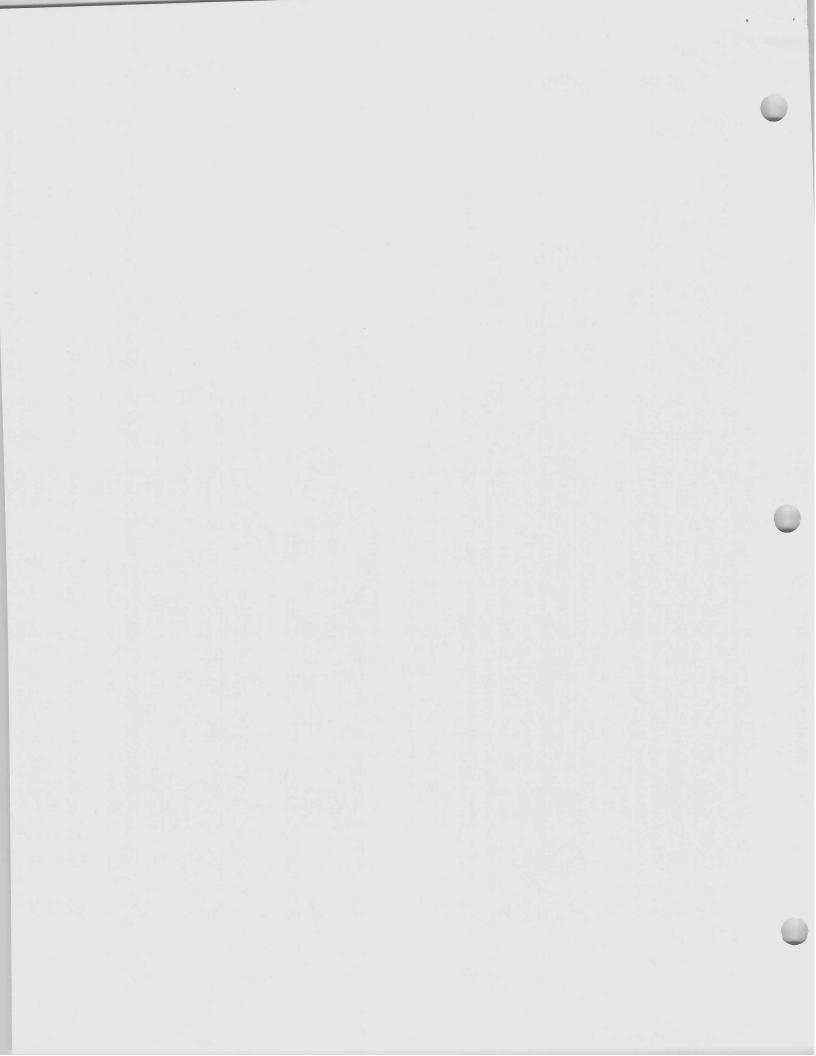
Interver 27

Inter

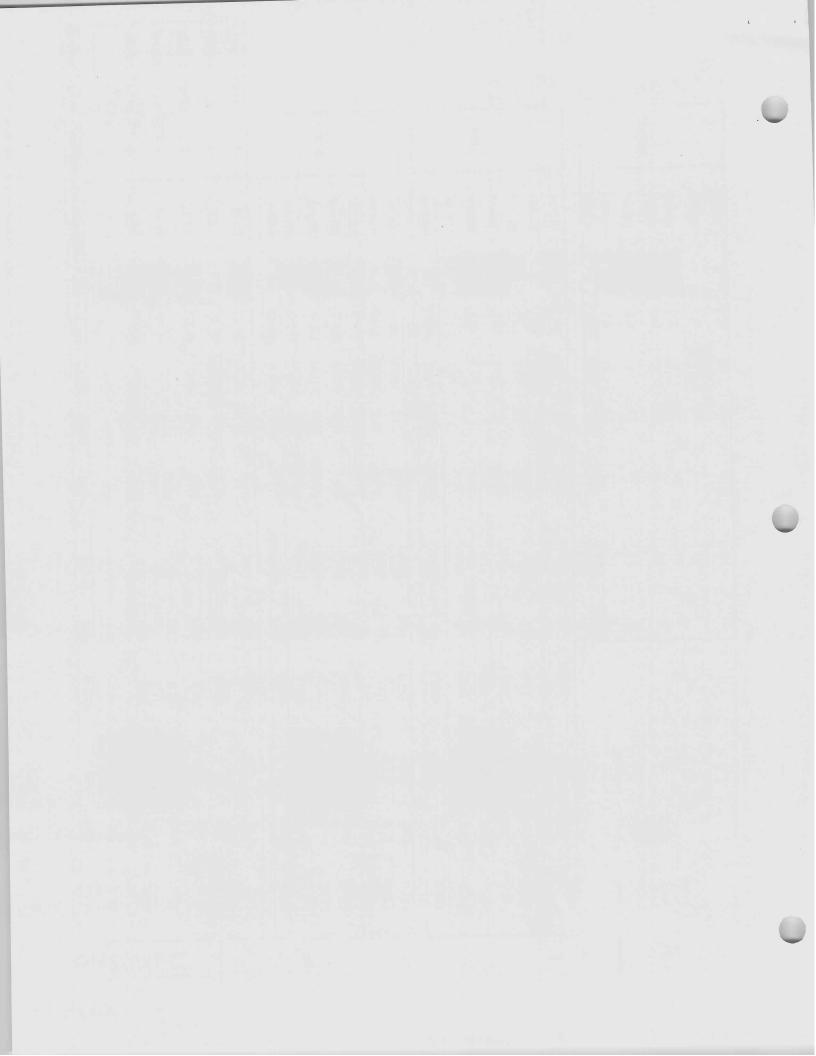
funning H20 @ 1:37 pm

@ 1:35 pm ¿ pause? immediatestop?

Normalized every cell by itself Vs paisson?



																								MD006		NDOSO	INF293	NF438	INE282	INEGOA
Γ								] [							7	Г							7	14.8	9.1/	0 17	10.23	24.087	2637	20.36
				CD107a								FIVIA								R10				1	<u> </u>	, L	2 / 000	, °1		Volume
NDOOR	ND030	NOCEO	INF293	INF438	INF282	INF694	Sample		ND006	ND050	INF293	INF438	INF094	Sample		MDOOD	NDOSC	NIDOTO	INF438	101238 2824NI	INF363	Sample	,	14.80	9.17	5.12	12.05	12.51	10.18	Con
6	σ	י	6	6	6	6	3M	1	2 1	U I	<b>)</b> 1	· ·	) K	>		0.80	0.6/	0.41	0.75	0.62	0./1	ME		0.20	0.33	0.59	0.25	0.38	0.29	1000
5.5	5.5	1 1	5 (	5.5	5.5	5.5	2.75M	1.00	1 82	1 00	1 E	1 83	1.83	2.75M		0.730	0.616	0.378	0.688	0.565	0.646	2.75M		0.19	0.30	0.54	0.23	0.35	0.27	2.75M
0	U	(	л (	л	ъ	5	2.5M	1.00	1.66	1.00	1.00	1.66	1.66	2.5M		0.664	0.560	0.344	0.626	0.514	0.587	2.5M		0.17	0.27	0.49	0.21	0.32	0.25	2.5M
4.5	4.5	;	л i	Л	4.5	4.5	2.25M	L.5	1 L	. L	1 1	1 1.5	1.5	2.25M		0.60	0.50	0.31	0.56	0.46	0.53	2.25M		0.15	0.25	0.44	0.19	0.29	0.22	2.25M
4	4	1	<b>1</b>	٠.	4	4	2.0M	1.32	1.32	1.32	1.32	1.32	1.32	2.0M		0.531	0.448	0.275	0.500	0.411	0.470	2.0M		0.14	0.22	0.39	0.17	0.26	0.20	2.0M
3.5	3.5	u.;	) U	) ( ) (	IJ Л	3.5	1.75M	1.16	1.16	1.16	1.16	1.16	1.16	1.75M		0.465	0.392	0.241	0.438	0.360	0.411	1.75M		0.12	0.19	0.34	0.15	0.22	0.17	1.75M
ω	ω	ω	ω	U	u i	ω	1.5M	1	₽	Н	Ь	1	1	1.5M		0.40	0.34	0.21	0.38	0.31	0.35	1.5M	0.10	0.10	0.16	0.29	0.12	0.19	0.15	1.5M
								0.83	0.83	0.83	0.83	0.83	0.83	1.25M		0.332	0.280	0.172	0.312	0.257	0.293	1.25M	0.00	0 0%	0.14	0.24	0.10	0.16	0.12	1.25M
			₽	<b> </b>	, ,	Volume		0.66	0.66	0.66	0.66	0.66	0.66	1.0M		0.265	0.224	0.137	0.250	0.205	0.235	1.0M	0.0	0 07	0.11	0.20	0.08	0.13	0.10	1.0M
			5.08	5.15	1 <u>1</u>	_ B		0.5	0.5	0.5	0.5	0.5	0.5	0.75M	0	0.20	0.17	0.10	0.19	0.15	0.18	0.75M	0.00	0 1	0.08	0.15	0.06	0.10	0.07	0.75M
		10.23	5.08	5.15			, and the second se	0.33	0.33	0.33	0.33	0.33	0.33	0.5M	0.100	0.133	0.112	0.069	0.126	0.103	0.118	0.5M	0.03	2 2	0.05	0.10	0.04	0.06	0.05	0.5M
			5.115		Average	1														100/1	0.66 m	0,33		200	0.66 (180)		3.32/1 <167	000	1 192 ( P)	13.00 4.00



AF647 hMR1 5-OP-RU 2	N M				FITC Va24Ja18 1.5	Va Mic			And UNSTAINED CONTROLS !!!											<b>21</b> 86	20 B4	19	<b>18</b> B3		16 V15	15 V14	14 V13	П	12 VIO		11 W	10 V5					6 U14	5 UVII	4 UVIO	<b>3</b> UV9	2 007		1 UV2	# Filter Single color (ul) Ref ctrl U
3 Tet:PBS	S		1/8	1776	15 Zombie NIR 0.8						APC/Fire 810 CD38	APC/Fire 750 CD27	Zombie NIR L/D	APC-R700 CD107a	AlexaFluor647 Va7.2/hMR1	APC CD16	PE-vio770 PD1	PE-Cy5 CD25	PE-Dazzie594 TNFa		e.c.k.		Ver							BV510 CD45RA	8V480 CD161	Pacific Blue CD19	Pacific Blue CD14	BV421 CD127	BUV805 CD4	BUV737 CXCR3	BUV661 V62	BUV615 CCR4				1		Unmixing ctrl Fluorochrome Marker
10 20 1_9 2_18					18		rippette draw volume /sample	or Media	Antibody Total		HIT2	0323	N/A	H4A3	3C10	3G8	P01.3.1.3	M-A251	MAB11	1011	BA5b	SK7	6811	11A9	827	M-T701	G043H7	5.1H11		HI100	HP-3G10	\$125C1	MSE2	A019D5	SK3	1C6/CXCR3	B6	161	FN50	RPA-T8		SK11	-	Clone Vial Lot #
<b>30</b>			Politica	6 Zombie			19.5	14.5	0.0	60			0.0	60																			1	1	1								1775	# During
ĺ		Nun	Nun	Nun	П		Pip	232	a a	3	-	1	2	96			-	-																									t	16
	Total	ber Intracellular SCs	Number Surface SCs (34)	nber Samples			Pippette draw volume /sample	Brilliant Stain	Antibody Total			1.200/	7500	<2:10/1.2>								/C.T /or.	c-10/1																				(8T) min @ RT	-
	0	30	-	16	70	-	ple 65	u,	1:	Towns I			-	.2>							-	.07							+	100						-			-	+	1		@ RT 30	mer 40
200		-	80		FBS	20000000	G	50 8	17.8 2	1.6 2	1					1980	1.2		1.5				4.0			2 1	,			2			1.5	1.5			2.0						30min @37C	HotCtain
2	2	0	0	28	1			800	285	25.6	32						19.2		24				1,4	24	TO	16	10	5		32			24	24			32						16	
7.8	0	0	3	4.8			59.5	50.0	12.5						0.7	1.5					1.2	2							0.7		2.0	2.0			1.3	0.7	1	0.5	0.7		1.2		30min @4C	2
14	1.2	6	2	4.8				800	200.0						11.2	24.0					19.2								11.2		32.0	32.0			20.8	11.2		8.0	11.2		19.2	1	16	
168	24	60	20	Perm 64																																							then Fix/Perm	RRC Lyse
1.87	0.15	0.75	0.25	PFA 0.72			19.5	E	9.5											2.5	0.1			1.5		0.7								0.5		0.1		1					Spiked 40 min @RT	
						L		176	152							8.0	8.0	24.0	8.0	40.0	1.6		No.	24.0		11.2	1.6							8.0		1.6		16.0					16	

Thaw cells, DNAss, count.
Collect, count, aliquot cells 2-3.0E+6 Cells R10 / Snd polystyrene tube
Bring volume up to "x" mit. R10 . add "y" jr. PMACtri and "x" jr. CD107a
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 5% PBS-FBS, spin 1300 rpm, 8min Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min Add HotStain mix, incubate @37C for 30 min

Add Tetramers, incubate @RT for 10 min Wash 2 ml 5% PBS.FBS 1400 rpm, 6 min

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

300 ul BD FixPerm, incubate @ 4C for 20min

(vortex every 10 minutes)

First PermWash: Second Perm Wash: 1 ml PermWash 1500 rpm 6 min 1 ml PermWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min
First PermWash: 2 ml PermWash 1500 rpm 6 min

Resuspend in 70 ul 0.4% PFA-PBS

Cap tubes, wrap rack in foil, store at 4°C

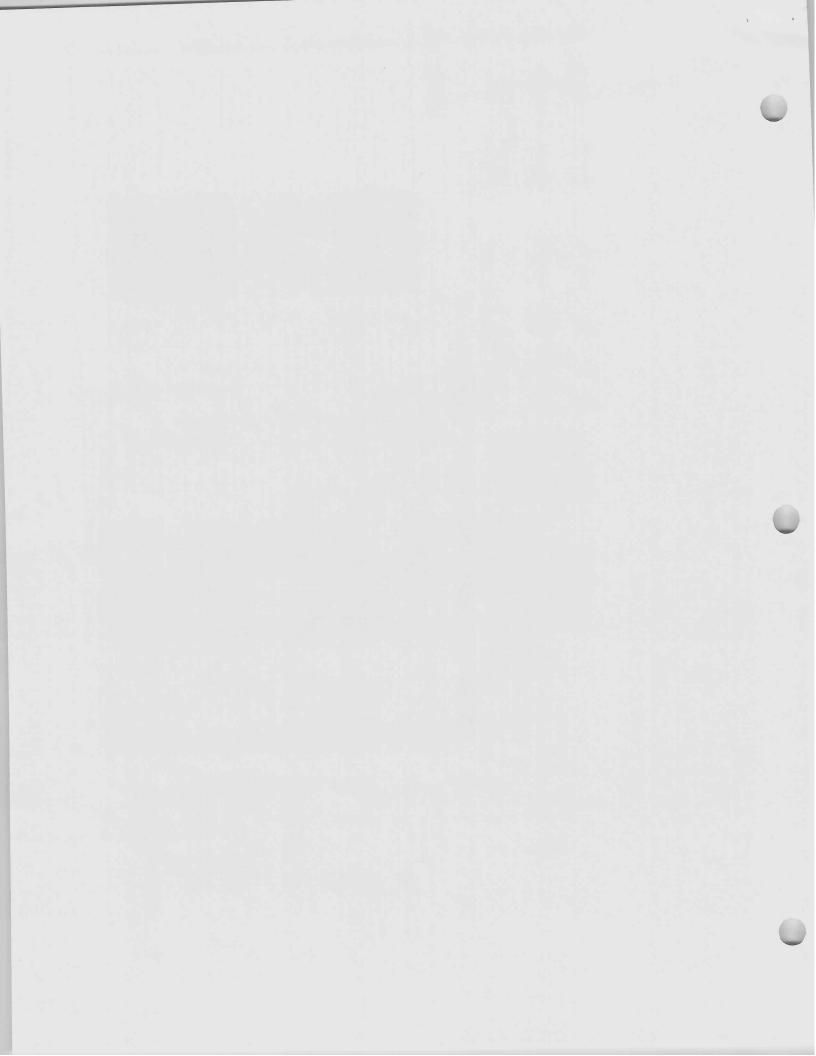
20 2\_18 2\_18

PFA 0.187 PBS 1.683 PBS

140

Perm Water RBC 0.78
Water 7.02

16.8 151



	812 UV16	783 UV15		750 UV14		717 UV13				598	11 7000	1275	020	514	308							Spectrum
		15				13 i	12	UV11	UV10		6An	8VU		UV7		DV6	DV5	UV4	. V3	UV2	CV1	_
	BUV805			BUV737				BUV661	BUV615		BUV563			BUV496		AF				BUV395		8
	[60]			1401				150	[45]		55)			[55]						(03)		
L	CD4			CXCR3				V82	CCR4		CD69			CD8						CD62L		
410	<16	V15	-	<u> </u>	V13	V12		V11	V10	§ ;	V8	5	6	;	<b>V</b> 5	<b>≤</b>	<del>≲</del>	<b>%</b>	<u>&lt;</u>			٦
		BV786	06/40	BV750	11.749			BV650	BV605	0.00	BV570	BV510			BV480	4	PacBlue		BV421			Violet
		1501			[55]			T .	150	SECTION AND ADDRESS.		(obs)		70-0	7,00		100		45		†	1
		200	IFNγ		CD7			CCR7	CDss			CD45RA		0	200		CD14/19		CD127			
B14	al a	B12		B11	B10	B9	B8	87	g G	84			3	B1							†	1
						PerCP-Cy5.5					Occ anion indo	FIIC/AF488									biue	
						[45]					foci										1	
					ı	CD26					CD3	Va24/hCD1d										
	YG9					Ę	YG5	YG3		YG1												
	Pe-Vio770					- Froys	DE-CV4	PE-Dazzle594		PE											Yellow-Green	
	1501							[60]		[50]												
	PD1					CDZS	CDon	TNFα		NKG2D												
	R7	<b>R</b>	ē	20 7	0 2	3 2	<u> </u>															
	APC-Fire 750	Zombie NIR		21.000	APC_R700	AF647	APC														Red	
	Ď	12.6		1	il a	E																
	CD27	Viability		00.00	CD1073	VQ/.Z/NINIK	CD16															

