2023 ILT 05

	Specimen	Status	Location	Conc	Data	NT .		2023_	LT_05	
COV	T consider		7 Bcx 3BA	< cont	Date	Notes	Volume	Lym	Lym+Mon	Total
193	0.0	12009	6	X6.97			1.0	8.91		
16000						The second secon	1.0	0-11	13.33	8,91
	a-5	RUQO	(10)	(11.37	, 3	r Plan	1.5	5.71	8.85	2.56
40/1 810 c.	Inf278	HEU-lo	BOX8AH	1	ber to			1	0.03	0.00
	Inf278	51-5	(H)	<21.77			0.5	4.52	11.1	
40/1 770 c.	a-2		60		-		A			
770 0	02	SLFF	0	121.97			0.5	4.43	10.5	
1749c.	Inf1595	HEU-hi	BOX GA AG	~ 3cm	4					
1070 C.	2-1	58FL	(3)	(11.57	14		1.0	8.64	12.3	- 71
SUIN			6	~3M					7	
	a-6	SBFIR	(B)	(13.87		and the contra	0.5	6.77	10.7	
11400.	a-8		60	~3M					4 4 4	
50/1		58 FM		<12.67			0.5	7.46	11.6	
6011	Inf0392	Heu-hi	BOX 2 BAY	~em		Liquid nitrogen sigide	a strings of	17.5	MEN LES LES	
920.	0-9		<u>(4)</u>	(28.17		Omassive clump o	1.0	2.75	3.05	
830c.	NDOSO	Adult	(2)	15EE	1/13/23					
11300.				(12.47		رط متحالي	1.0	8.49	11.6	
	ND006	Adult		15E6	5/12/23				1 1	
MOLL, T				6.97			1.0	15.10	18.0	
112	Gan Than	1 6		1				1		

lifepon Them short spin ellist DNASCELLES 11:54 -11:36 am is sock set 50.0 11:50 12:10 PMB (es 012:11 11:53 an Lad cord set spin @ 12:00 pm 12:26 DWASA Stain count @ 12:46 pm

Count @ 1:00 pm

3rd cerd set spin@ 2:05pm 2:18pm DNAse

2:36 pm algors

3:12 pm mixes prepred

3:27 pm Incobation start > 9:27 pm

Research propped @ 8:27pm

9:16 ABS prepped

9:33 sample spM

1/De 9:47 -> 10:02 pm game 10:00

5c 9:53

10:11 50 40 7 36

10:17 horses > 47 spine Hopm

10:26 samples hot 750 Spine 11:02pm 10:36 cold scs -> 11:06

<16,45 Tet Mixes made >

Tets @ 11:18pm -> 11:59pm

Sc FK Perm @ 11 25 pm -> 35 -> 45 pm

Int 278 7B CS-C7

INS 039 ZA 64

YRUTG as

00 00010 0000 00 000 00 0010 00 0010 00

000

15t Pemmas (Scs C1149 pm

combined spin @ 12:07 am

12:24 pm cold samples -> 12:54 12:30 am Intrascs > 1:10

Rbelysee 12:57 am spin @ 1:04 am

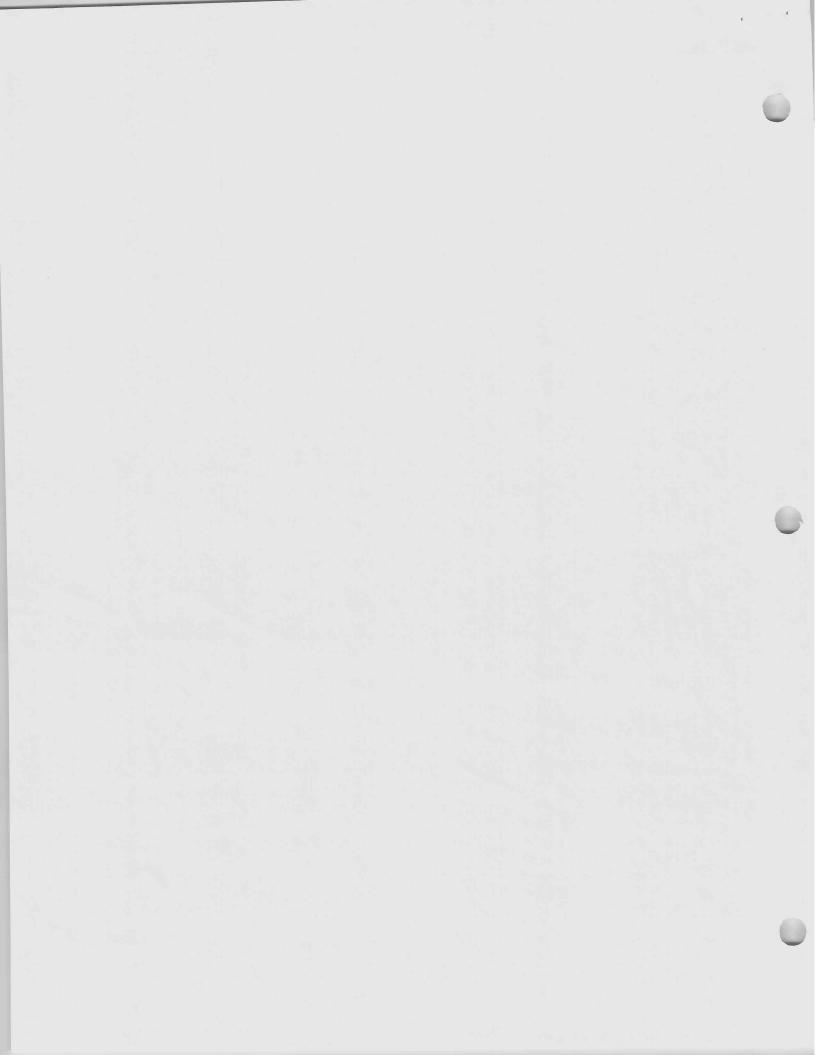
FILPER @ 1:18 728-7 38an Sis done @ 1:22 pm

permand spine 1:56 am.

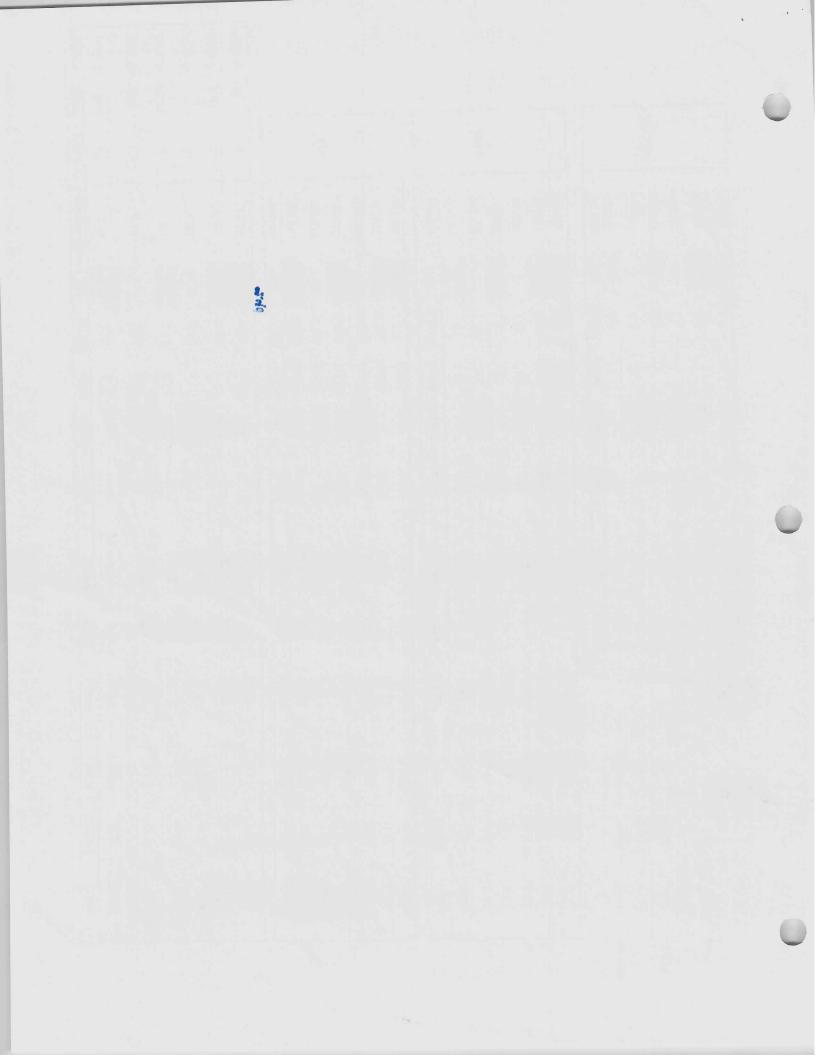
2:09 Intracellar -> 2:49km First spin @ 2:50 am Done @ 3.01 am

12:56 stert Inf 159 Inf039 Inf 278 Infoss (Indspeamen 1.75 Rog; samples Info39 was within ballparti runge # lymphocytes. . HOIR 2:11 final sample. Sc's C 2 (14) 3800/50 -> 280,000 erats 3/42 Violets 8/44/6/54

M



																							_							
																							ND006	ND050	INF039	INFLS9	INF278	INFUSS	ואודסדי	Sample
													-		Г							7	15.1	8.49	7	15./6	6.72	17.48	1	Total
			2	CD107a							PMA								R10				Ъ	Ь	2	2	1.5	2.5	o diamin	Volume
ND006	ND050	INF039	INF159	INF278	INF055	Sample	NDOOB	ND050	INF039	INF159	INF278	INF055	Sample		ND006	NDOSC	NIDOEO	INF159	INF278	INF055	Sample		15.10	8.49	3.50	7.88	4.48	6.99	concentration	
o	6	6	6	6	6	3M	2	2	2	2	2	2	3M		0.80	0.65	0.14	0.62	0.33	0.57	3M	Dh'Le	0.20	0.35	0.86	0.38	0.67	0.43	SIVI	
л Л	5.5	5.5	5.5	5.5	5.5	2.75M	1.83	1.83	1.83	1.83	1.83	1.83	2.75M		0.734	0.592	0.130	0.567	0.302	0.523	2.75M	1	0.18	0.32	0.79	0.35	0.61	0.39	2./5M	, , , ,
л (U)	G	υ	Сī	٠.	2.5M	1.66	1.66	1.66	1.66	1.66	1.66	2.5M		0.667	0.539	0.119	0.516	0.275	0.475	2.5M		0.17	0.29	0.71	0.32	0.56	0.36	2.5M	
ъ . п (4 5	4.5	4.5	4.5	4.5	2.25M	1.5	1.5	1.5	1.5	1.5	1.5	2.25M		0.60	0.48	0.11	0.46	0.25	0.43	2.25M		0.15	0.27	0.64	0.29	0.50	0.32	2.25M	
, 1	7	4	4	4	4	2.0M	1.32	1.32	1.32	1.32	1.32	1.32	2.0M		0.534	0.430	0.095	0.412	0.220	0.380	2.0M		0.13	0.24	0.57	0.25	0.45	0.29	2.0M	
, ω. 	ა •	3.5	ω	3.5	3.5	1.75M	1.16	1.16	1.16	1.16	1.16	1.16	1.75M		0.467	0.377	0.083	0.361	0.192	0.333	1.75M	0.14	0 1 2	0.21	0.50	0.22	0.39	0.25	1.75M	
u	، د	ω (ມ	ω	ω	1.5M	1	Ъ	1	Ъ	Ь	1	1.5M		0.40	0.32	0.07	0.31	0.17	0.29	1.5M	0. TO	0 10	0.18	0.43	0.19	0.33	0.21	1.5M	
		ر ائر					0.83	0.83	0.83	0.83	0.83	0.83	1.25M		0.333	0.269	0.059	0.257	0.137	0.237	1.25M	0.00	0	0.15	0.36	0.16	0.28	0.18	1.25M	
エル	í	۲	٠ د	Ь	Volume		0.66	0.66	0.66	0.66	0.66	0.66	1.0M		0.267	0.215	0.047	0.206	0.110	0.190	1.0M	0.07	0 07	0.12	0.29	0.13	0.22	0.14	1.0M	
I PAA (0.04	0 6/	7.12	Lym		0.5	0.5	0.5	0.5	0.5	0.5	0.75M		0.20	0.16	0.04	0.15	0.08	0.14	0.75M	0.05	2	0.09	0.21	0.10	0.17	0.11	0.75M	
3 in 2 Parts	7 2000	0.04	0 67		Total		0.33	0.33	0.33	0.33	0.33	0.33	0.5M		0.134	0.108	0.024	0.104	0.055	0.095	0.5M	0.03	3	0.06	0.14 110	0.06	0.11 80	0.07	0.5M	
6414		1.0/0	7 070		Average									_																



1.5 24 24 24 24 24 24 24 2	96 <1.2500> <2.511 96 Antibody Total 232 Brilliant Stain	6.0	Antib		And UNSTAINED CONTROLS !!!
1.5 24 1.3 1/1.5> 24 1.2 1.9.2 0.1 1.5 24 1.2 19.2 0.5 1.2 19.2 1.5 24.0 0.5 1.2 19.2 1.5 24.0 0.5 1.5 24.0 0.5 0.5 1.5 2.0 0.7 11.2 1.6 25.6 1.5 20.0 9.5 1.7.8 285 12.5 20.0 9.5	<1:2500> Antibody		Antik		NSTAINED CONTROLS
1.5 24 2.5 2.5 2.5 2.5 2.5 2.5 2.5 2.5 2.5 2.5	41.2500>	6.0			
1.5 24 2.5 1.2 19.2 0.1 1.5 2.4 2.5 1.5 2.4 0.5 1.5 1.5 2.4 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5	41:2500>	6.0			
1.5 24 0.1 1.2 19.2 0.1 1.5 24 0.5 1.5 24 0.5 1.5 24 0.5 1.7 19.2 0.5 1.7 11.2 0.5	41.2500>	6.0		APC/Fire 810 CD38	29 R8
1.5 24 1.2 19.2 0.1 1.5 24 2.5 1.5 24 0.5 1.5 24 0.5 1.5 24 0.5 1.7 19.2 1.5 24.0 0.5 0.7 11.2 0.5		6.0			20 (1)
1.5 24 1.2 19.2 0.1 1.5 24 2.5 1.5 24 0.5 1.5 24 0.5 1.5 1.5 24 0.5 1.7 11.2 0.5 0.7 11.2 0.5					
1.5 24 1.2 19.2 0.1 1.5 24 0.5 1.5 24 0.5 1.5 24.0 0.5 0.5 0.7 11.2 0.5				APC-R700 CD107a	1
1.5 24 1.2 1.2 0.1 1.5 24 0.5 1.5 24 0.5 1.5 24 0.5 1.5 24 0.5 1.5 2.5 1.5 2.6 1.7 0.5 1.8 0.5 1.9 0.5 1.9 0.5				AlexaFluor647 Va7.2/hMR1	
1.5 24 1.2 19.2 0.1 1.5 24 0.5 1.5 1.5 24 0.5 0.5			- Carrier and Carr	APC CD16	
1.5 24 1.2 19.2 0.1 1.5 24 0.5 1.5 24 1.5 1.5 24 1.5 1.5			PD1.3.1.3	PE-VIO770 PD1	24 R1
1.5 24 1.2 19.2 0.1 1.5 24 0.5			M-A251	PE-Cy5 CD25	23 B13
1.5 24 1.2 1.2 0.1 2.5			MAB11	PE-Dazzie594 TNFa	
1.5 2.4 And 1.2 19.2 0.1			1011		
1.5 24			BA5b	reice-cys.5 CD26	20 B4
1.5 24			SK7		19
C-T	0		51d 6811	Va	18 83
			11A9		17 B2
			B27		16 V15
			M-T701	CD7	15 V14
					14 V13
30			5.1H11		13 V11
7.11					12 V10
0.7			ні100	CD43KA	
					11 V7
20 320				BV480 CD15	
30					V3
20.5 8.0					9 V3
30					√1 ∞
1.3 20.8					7 UV16
0.7 11.2 0.1 1.6			106		6 U14
				BUV661 V62	
0.5 8.0 1 16.0				BUV615 CCR4	
0.7 11.2				BUV563 CD69	
			RPA-T8	BUV496 CD8	3 UV9
1.2 19.2			JV6	AF AF-UV6	2 UV/
			SK11	BUV395 CD62L	
Tetramer 40 HotStain CoddStain RBC Lyse, Spiked 40 min 16 then @RT 16	16 (RT)	Vial Lot # During stim!!!	Clone	name Fl	1 UV2

Simplified Protocol

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 Thav cells, DNAse, count.

Collect, count, aliquot cells 2.3.0E+6 Cells R10 / 6ml polystyrens tube.

Bring volume up to "x" mL R10, add "y" µt PNIA/Ctrl and "z" µt CD107a Cap and incubate at 37°C for 6 hours

Add HotStain mix, incubate @37C for 30 min Wash 2 ml 5% PBS-FBS 1400 rpm, 6 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

PBS Pippette draw volume/sample:

Zombie NIR 0.8

14.4 15 PBS 5.76 6 Zomble

1.2

64 PFA 20 20 24 168

1_9

 20
 30

 2_18
 3_27

 2_18
 3_27

 3_18
 3_27

20 2_18 2_18

PFA 0.187 PBS 1.683 FBS 140 PBS 140

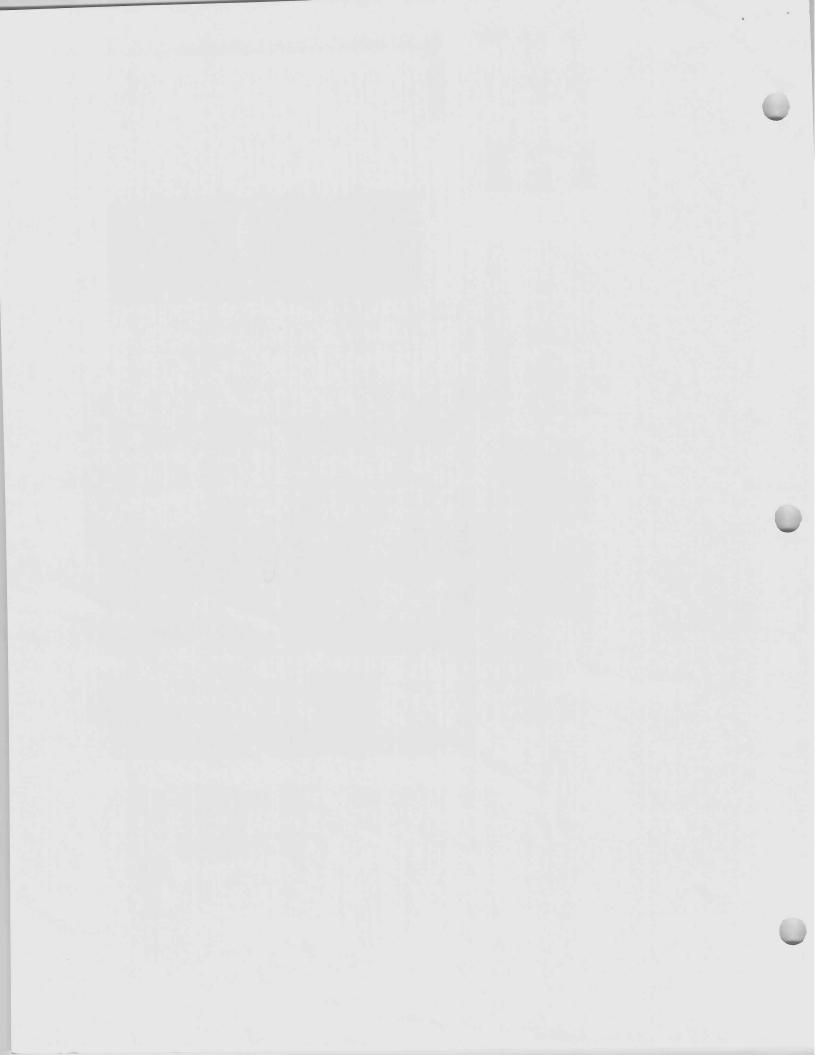
RBC 0.78
Water 7.02
 Perm
 16.8

 Water
 151

Tetramer Control Mix

AF488 hCD1d Unloaded

AF647 hWR1 6-FP



	-	760	738								525		000				428	388	373	Spectrum
UV16	UV15	UV14		UV13	V12	UV11	UV10		600	8VU	_	UV7		0V6	UV5	UV4	UV3	UV2	LY1	
BUV805		BUV737				BUV661	BUV615	!	BUV563			BUV496		AF				BUV395		VV
[50]		[40]		,		1501	[45]		[55]			155						[50]		+
CD4		CXCR3				V82	CCR4		CD69			CD8			,			CD62L		
V16	V15	V14		V13		V11	V10	6	8	5	S6	į	V5	≨	₃	<u>~</u>	<u> </u>		1	1
	BV786	BV750	:	BV711		BV650	BV605		BV570	BV510		1,00	BV480		PacBlue	į	BV421			Violet
	(50)	feet	lood.	n n		1501	501	SCOPPING NO.		H		7.0	55		[55]		45		\dagger	1
	CCRS	IFΝγ	5			CCR7	CD56			CD45RA		CD.19.1	2262		CD14/19	77.00	CD197			
B14	B12		B10	B9	B	B7	9 6	B 5		B 2		<u>B</u>							† .	1
				PerCP-Cy5.5					Chair Prine 220	FIIC/AF488									biue	
				[45]					Inci					11.0						
				CD26					נטג	Va24/hCD1d										
YG9					YG5	YG3		YG1												
Pe-Vio770					PE-Cv5	PE-Dazzle594		PE											Yellow-Green	
[50]					b	[60]		[50]		****										
PD1				i d	CD25	TNFα		NKG2D												
R7	R6	R5	R ₄	23 23	3 3															
APC-Fire 750	Zombie NIR		APC-R700	3	AF647)													Red	
[45]	[55]		55		<															
CD27	Viability		CD107a	VIII (7: 10 A	CD16															

