1/00-	Specime	n Status	Location	n Conc	Date	Notes		2023	_ILT_14	
1500c	Inf 105-	-6 HU 6	7		Date	Notes	Volume	Lym	Lym+Mon	Total
1350C	a	RYKG 5-6 HU &		3) < 6.277			1.0	12.5	19.9	512
1460cm	a	2 RYKT	a	(6.57		7	)"	12.2	18.9	
60y   1310c;	a	2 RW7M 8	(b)	eg.007		nikayan		8.38	12.3	
60 / Szoc.	0-1	RW7L	10	48.827		nidiogen	[	7.55	11.3	
4011	Ins 041-5	RUWY	(5)	<12.47		nitrogen	1	6.94	11.6	
1410c,	041-5 a-2	HEU-10 9 RUWZ		13.187		nitrogen	1	6.70		
920 0	Inf 12:4-7 a-4	HEV-N: 27 S6FK		(8.37>		bloody lotalls	1.5		10.3	
						g		6.76	10.4	
90 c.	Inf235-0									
700.	a-1 Inf 1350	SCSY HEU-Ni ?	0	21.05>			0.5	3.01	4.84	
170	2-20	5C5 Z	13 <	17.077			0.5	3.54	5.63	
101		Trwa	<b>(1)</b>		1	Lysed Lysed	1.0			
1 1401	Inf647-1	HRUHIEG TKUR	(E)		)	Ysed ?Fully Lysed	1			- 2
200	1	Adult 8	3	20 E 6 1/19	3/23	(1 P. 3 5 L.	1	9.42	12.90	
ETT	10000	Adult 7			12/23		1	15.1	18.40	
1	@ 10:20	an 10:32 51			10					

1			
Thaw @ 10:20 an	10:32 5 pin	So drove	11:18 1029
	10.48 pm 58	IT 11:21 dance	1120 45
12:03 pn! st	ain for	11:165 pm 11:	39 dnase
count done, wa			

1:00 pm aliquot forst

2:05 pm Incubation start > 8:05 pm

< Antibody prep@ 5:15pm> Reagents propped @ 6.30 pm

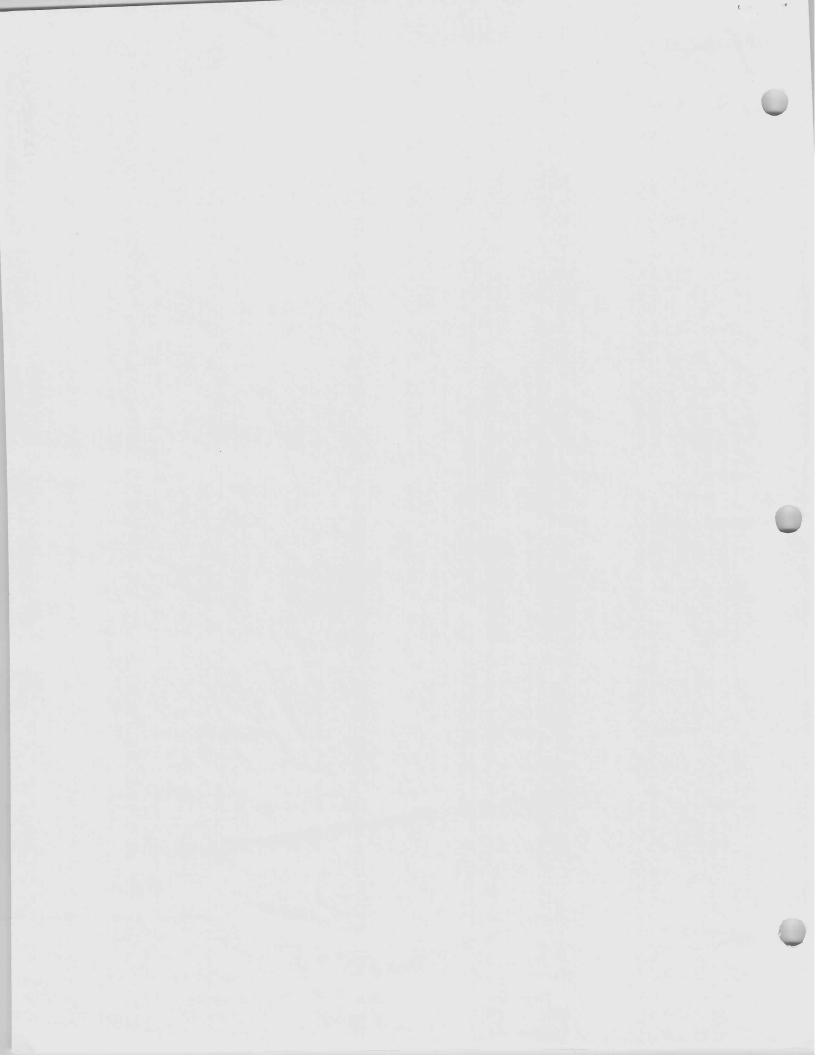
68:06 SC Spins 8: 24 sc hot -> 8:54 pm - spine 8:55pm WOE 8:30pm -> 45 -> Spine 8:52pm 8:38 se cold -> 9:08 pm -> Spine 9:15 Tets propped @ 9:00 pm Het samples eq: 11 pm - 79:41 pm/

0	000	0	06	
0	<b>100</b>	0 0	00	
0	00	0	00	00
0	00	0		000

1:27-7:37-7:47 pm Se's 1st wash @9:52pm 2nd washe 10:05 pm

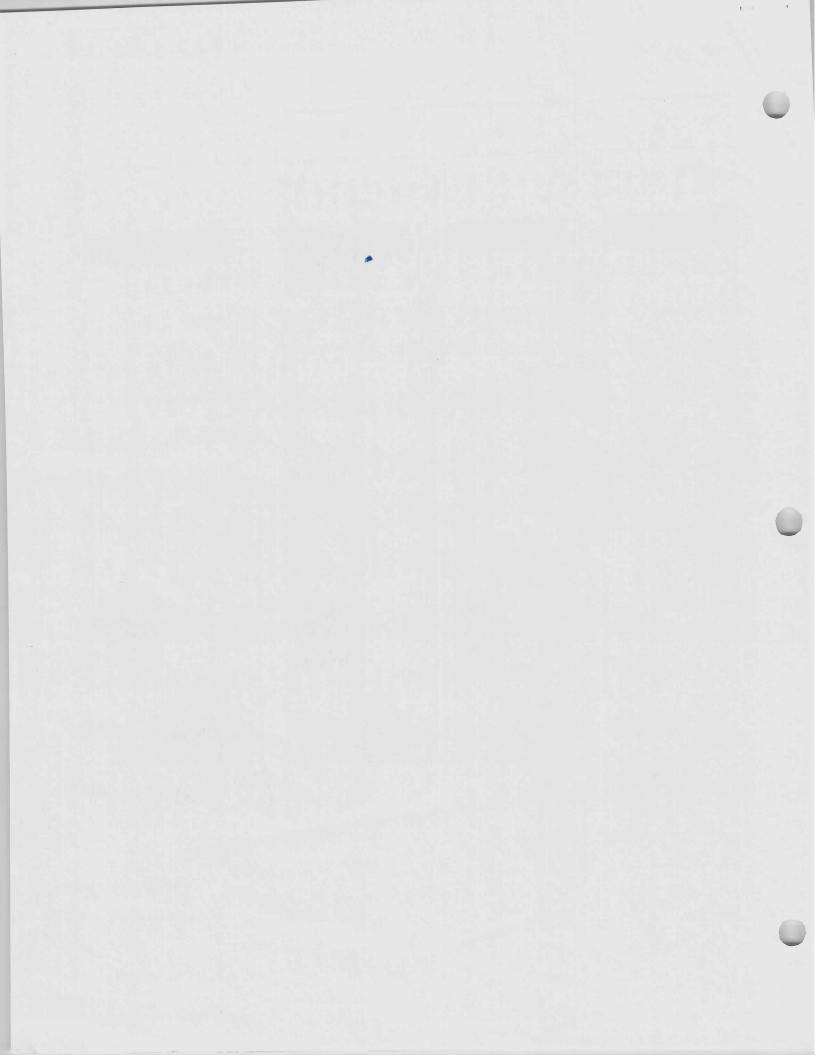
10:08 tets -> 10:48 pm 10:18 Vas > 10:43 10:22 mfasc's -7 11:02 pm Bulkschin15 pl@ 10:31pm Spin @ 10:54 pm Cold somples @11:10 -> 11:40 pm 30's done @ 11:16

cold -> 11:40, RBC lyse (	= 11:47 pm	> 11:30pm			
Spin @ (1.54 pm					
Fix Pem @ 12:09-7:1	9->:29				
1 st mash @ 12:33					
2nd rush @ 12:45					
12:59 intracellular s	tert				
Unstarted's in 15pl G	2.03 pm				
Done @ 1:580	in /				
	ny ari-ta-damin'ny ari-tanàna amin'ny ari-tanàna amin'ny ari-taona ao amin'ny aritana amin'ny aritana amin'ny				
1/QC@5:35 pm// issues 4/ beard	concentration	2 failed s	starts		
Reflib starte 5:47p	l'oa		Accor	DNs hipper	
done @6.41	W.			ted HAS?	
				Cols:	0
Grabbing coffee,	will be r	ight back	!!		
		26:41pm			
7:00 pm	1	(~)		1 3	1
0 /			1/1	12	
0 100		, 0	1000	100	10
	7:10pm	Tabili	7 ( 77)	2= 0 = =	
		Inful	124 7.00	"Inf235 7"	my o
		2311/2911	26K 4.511 31 H 2.2 M	27.8M/5.5	-/ /.0
1.8M 45pl 38p	1? 1.9m! 3	36/1 ~1.6M	37 H 2.2 M	43 / 36m	37/ 16
Despite bene	, oldest, he	althrest?	Encudo ci	045 - hour	<i>' ' ' ' ' ' ' ' ' '</i>
to a rillian	1		speci	wers factor d	
Done @ 7:46pm					



	_		_			_	-5-		_	7	Г										1 -											ш
-					DT0/4	D1075										PMA										R10						Д
NO		NDOOG	NOTO	INF235	INF124	INFO41	INEO/1	INEOE /	INF105		ND	MDOOR	NDOSO	NIPOTO	INFT24	14101	INFO04	INIEUGA	INE105	Sample	N	NOOD	NDOSC	NIDOFO	INIT 25	INF041	111004	INIEURA	INF105	Sample	-100	1.00
0	0	0 0	1	6	6	σ	) 0	) (	SM SM		2	2	2	2	2	) /	) K	2 ~	SIN	3	-2.00	1984	7.68	1,5	0.56	100	797			ω 	100	2 00
5.5	5.5	1 5		5.5	5.5	5.5	i 5.5	י נ	2.75M		1.83	1.83	1.83	1.83	1.83	1.83	1.83	1.83	2.75M		-1.834		0.68 5 40.624	0.08 20.075	0.56460.509	0.56160.513	V62 520.5/1	97.000.693	10000	2 7EM	2./3	
5	G	5	ţ	л	5	5	U	U	2.5M		1.66	1.66	1.66	1.66	1.66	1.66	1.66	1.66	2.5M		-1.667	0.667	0.568			0.466	0.519		-		2.50	
4.5	4.5	4.5	į	Л	4.5	4.5	4.5	4.5	2.25M		1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	2.25M		-1.50	0.60	0.51	0.06	0.42	0.42	0.47		1.		2.25	
4	4	4	4	<b>.</b>	4	4	4	4	2.0M		1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32	2.0M		-1.334	0.534	0.454	0.054	0.370	0.373	0.415	0.504	2.0M		2.00	
ω	ω.5	3.5	ω 51	) (	ω 5	ω <u>.</u> 5	3.5	3.5	1.75M		1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.16	1.75M		-1.167	0.467	0.397	0.048	0.324	0.326	0.363	0.441	1.75M		1.75	
JJ	ω	ω	ω	(	υ ——	ω	ω	ω	1.5M			Ь	1	ш	ш	Ь	ы	ъ	1.5M		-1.00	0.40	0.34	0.04	0.28	0.28	0.31	0.38	1.5M		1.50	
										0.00	0 83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	1.25M		-0.834	0.333	0.283	0.034	0.231	0.233	0.259	0.315	1.25M		1.25	;
					į	0.5	0.5	Volume		0.00	0 66	0.66	0.66	0.66	0.66	0.66	0.66	0.66	1.0M		-0.667	0.267	0.227	0.027	0.185	0.186	0,207	0,282	1.0M		1.00	0.0
					;	3 54	3.01	Lym		0.0	o c	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.75M		-0.50	0.20	0.17	0.02	0.14	0.186 /36 0.14	570.16	0,282 2020.19	0.75M		0.75	0.00
				3.2/5	777	1 77	1.505	Total		0.55	0 0	0 33	0.33	0.33	0.33	0.33	0.33	0.33	0.5M		-0.333	0.134	01446	0.014	0.093112	0.094	0.104	0.127	0.5M		0.50	0.00
					1.000	1 638		Average		L										L		- 1			N					· 26.	12	S.

Sample	Total	Volume	Concentration	3M	2.75M	2.5M	2.25M	2 0M	1 7500	1				
INF105	24.7	>	د د ت					T.0141	T./SIVI	T.SIVI	1.25M	1.0M	0.75M	0.5M
	:	١	12.35	0.24	0.22	0.20	0.18	0.16	0.14	0.12	0.10	0.08 0.06	0.06	0 04
INF064	15.93	2	7.97	0.38	0.35	0.31	0.28	0.25	0 22	5	2		)	
INF041	13 64	د					į	0	0.22	61.0	0.16	0.13	0.09	0.06
NF124	10.04	, ,	6.82	0.44	0.40	0.37	0.33	0.29	0.26	0.22	0.18	0.15 0.11 0.07	0.11	0.07
	1	7.5	6./6	0.44	0.41	0.37	0.33	0.30	0.26	0.22	0.18	0 15	011	0.07
INF235	3.27	_	3.27	0.92	0 84	0.76		)				,		0.0.
ND050	9.42				-		0.03	0.61	0.54	0.46	0.38	0.31	0.23	0.15
NDOOR	1	+	9.42	0.32	0.29	0.27	0.24	0.21	0.19	0.16	0.13	0.11	0 08	0.05
	1.61	<u>-</u>	15.10	0.20	0.18	0 17	0 15	2						Section 2
ND	Ь		- 80			0.1	C.	0.13	0.12	0.10	0.08	0.07	0.05	0.03
			1.00	3.00	2.75	2.50	2.25	2.00	1.75	1.50	1.25	1.00	0.75	0.50
														0



Areas         hChd Unbaded         0.5         0.6           Areas         hMR16FP         2         2.4		AF647 hMR1 5-OP-RU 2 12	1.2   1.2   1.5   17.8   1   1.2   1   1   1   1   1   1   1   1   1	Va Mix         11           FITC         Va24la18         1.5         16.5           AF647         Va2         1.5         1.5		CONTROLS !!!	And UNSTAINED CONTROLS		<b>29</b> R8		20 R4 27 R6		24 RJ		22 88	20 B4 21 R6		<b>18</b> B3		1	1	14 VI3	13 VII		11 V/	10 V5		SO VI		0 014				2 ()V/		# Filter Single color (ut) Ref ctrl Unmixing ctrl name
Tet		Te		Zombie NIR				,	APC/Fire 810 CD27	Zombie NIR L/D	APC-R700 CD107a	AlexaFluor647 Va7 2/hm	70		PE-Dazzie594 TNFa		PerCP-Cy5.5 CD26	Va					BV605 CD56	CD45RA		ie	ie							8	AF C	Fluorochrome
10 Tet:PBS 1 9 Tet:PBS 1 9	1.9	10 TetP8S 1_9	6.08	19	Pippette draw volume /sample	R10 Media	Antibody Total	HIIZ		N/A	a H4A3				a MAB11						M-T701			SRA HI100	161 HP-3G10			CD127 A019DS			V82 B6		CD69 KPA-T8		CD62L SK11	
20 30 2_18 3_27 7_18 3_27		<b>20 30</b> 2_18 3 27	7 Zombie		19.5	14.5	6.0 1			0.0																										Vial Lot # During stim!!!
PFA 0.156 PBS 1.404	FBS PBS	Total	Number Samples Number Unstained Number Surface SCs (29) Number Intracellular SCs		Pippette draw volume /sample	246.5 Brilliant Stain	102 Antibody Total			<1.2500>								<:10																		17 L/D 15 min Tet
	108 Perm 108 Water		17 136 11 44 30 30	Total	sample 65	<b>50</b> 850			2 34		<2:10/1.2>		1.2 20.4		1.5 25.5			<:10/1.5>		1 17	1 17	1.0 17		1	ن		1.5 25.5				2.0 34					Tetramer 40 HotStain min @ RT 30min @ 37C 1
0.84 7.56		8.4	5.1 3.3 0	8	,59.5	50.0 850	12.5 212.5	2				0.7 11.9	1		5		1.2 20.4		n si		7	7		0.7	2.0		.5	.5	1.3 22	0.7 11			0.7		Y	17 ColdStain 30min @4C 1
		1.2 24 14.5 174 156.6	5.1 68 2.2 22 6 60		12											1								11 9	34.0	34.0			22.1	11.9		8.5	11.9	0.4	20.4	17 RBC Lyse, then Fix/Perm Spik
		0.12 1.56	0.51 0.33 0.6		19.5		9.5 161.5					8.5			1	1	0.1 1.7		<u>1.5</u> 25.5			0.1 1.7						0.5 8.5		0.1 1.7		1 17.0				Spiked 40 min
						Cap tubes, w		Resuspend	First PermW	Add Intracell	second Pem	First PermW	1	1 22		390 UI DU TI	300 80	Wash 2 ml 5	Add 300-500	Add ColdSta		Wash 2 ml 5	Add Tetram	Wash 2 ml 5	Add HotStai		Wash 2 ml 5	800 ul of Liv	Wash with		Cap and inc	Bring volum	Collect co.	!		Simpli

## Simplified Protocol

w cells, DNAse, count.

Bett, count, aliquot cells 2-3.0E+6 Cells R10 / 5ml polystyrene tube lect, count, aliquot cells 2-3.0E+6 Cells R10 / 5ml and "z" μ CD107a g volume up to "x" mL R10, add "y" μ PMA/Ctil and "z" μ CD107a and incubate at 37°C for 6 hours

and incubate at 37°C for 6 hours

with 2 ml PBS, spin down 1000 pm 8min

ul of LiveDead mix (1:2500) @RT for 15min

ul of LiveDead mix (1:2500) @RT for 15min

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

dd ColdStain mix, incubate @ 4C for 30min dd 300-500 ul 1x RBC Lysis for 3 minutes ash 2 ml 5% PBS-FBS 1400 rpm, 6min

I BD FixPerm, incubate @ 4C for 20min

(vortex every 10 minutes)

1 ml PermWash 1500 rpm 6 min 1 ml PermWash 1500 rpm 6 min

wash. Tilli Felliwadsii Isoo ipin 6 min

acellular Stain, incubate @ RT for 40min mWash: 2 ml PermWash 1500 rpm 6 min

Resuspend in 70 ul 0.4% PFA-PBS

ubes, wrap rack in foil, store at 4°C

0

E 1

