	March 21st,	2023							2022	) II T		
	Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly		3_ILT_0:		
601/	Inf0325	HUF		=					Ly+Mon	Total	3E+6	.3E+6
Kl1	a-4		(3)	416,457	CB/0 3/10		1.0	5.79	7.52			
A A	0.3	RRFJ	(3)	613.377			1.0	5.06	6.84			
YM	Inf100-1	HEU-JO RYES	(A).	47.747			10	4.62	8.74			
17	a-2		6			< Dead 7	0.5	0.19	CARRY CARRY			
AFK.	Inf314-8	HEUNI	(5) e	-127			1.5	6.22	9.00			
9/1	1 04	SNHD	$\widehat{\mathcal{T}}$				1.5	6.90	9.64			
. "	ND05G	Adult Norm	0	BE6	1/13/23		1.5	5.37	7.37			
	NDOOG	Adult ses		15E6 1	120/23		1.5	7, 24	9.13			
i V	Man @ 10:30 a		•				1.0	6.79	11.5			
	Final there: 1						T.	~ ( \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	2.4			
	11:3e 3rd SWAS					Colgi Bah	500K	1+ 03	2: 10.85 0: 11.61	5 % 2	9+	1,5=10.5
	stan e 11:										_	K1.5= 10.
-	12:07pm	1				34	s s In	if 314	1: 13012	ME	∌	
		<del></del>				\v'	A, , A	<b>&gt;</b> -		3 % 3		
	ncubation	@ 2:	19 pm /	781	9 pm	· ·		0020	. 8.05	Z. 1.:	5   5.	37
Pa	agents.	0.5.2.2.1	17.00		h!		<u></u>		10.86		1	24
Tree		he chos	a @ 8) (	(6			Sa., 0	les T	2Bc 1		The state of the s	
L	mples spi	n In 1 A	35 C 8:	C6 pm	í		early		CDC lyse	@ 12	:06 an	1
	pe 8:4	3 pm	-7 59	3 pm					ZBC lyse Final fo	35 wa	sh a	~ : ~
	Massive.	spin E	O GIVI CO A	-				T	0		0	0.13

Samples spin la 1 PBS @ 8:06 pm

L/DO 8:43 pm -> 58 pm

Massive spin @ 9:04 pm -> 9:12 pm

Hot samples a q:30 pm for 10:00 pm Washe 1608

3PC seis @ 01400 m -> 10:00 pm Washe 1608

Hot samples a 2:30pm & 10:00 pm Washe 1608

3Pc seis e 9:40 pm -> 10:10 pm Spine 10:16 pm

4° es e 9:53pm -> 16:23 pm Spin @ 10:28 pm

Tets e 10:24 ->

Va7.2010:36 pm - 11:05pm/ Cells @ 10:43 pm -> 53 -> 11:03pm Combined spin @ 11:16pm > 22 4°c @ 23:30pm -> 00:00 Sc's 2nd Perm @ 11:38pm Intrascis C 00:03 -> 00:43 Fix Perm @ 12:24-734-744

1:16 pm intrac > 1:56 am

Final spin @ 2:01 am

Done @ 2:13 an

Ba 1-001 201

Took ela

•

																			NUCCO		NJ OF O	INF314	INF100	000	INEOSO	Sample
			7	Г						_	ır			-					10.86	3 9	о Оп	19.68	11.61	10.63	10 OL	Total
CD107a							PMA							710	B10				1.5	1.0	٦ (	n	2.5	~	۵	Volume
INFLOO	INFOSZ	Sample	Camala	NDOOD	NDOOG	NDO50	INE314	INICOS	Jampie	Commul-		NDOOG	NDO50	INF314	INF100	INF032	Jailiple	Sample	7.24	5.37	1 C	л Л	4.64	5.43	1	Concentration
6	σ			7	, v	) V	) V	, v	, u		0.00	0 70	0 0	0 54	0.35	0.45	SIVI	200	0.41	0.56	0.40		0.65	0.55		3M
5.5	5.5	2.75M		1.83	1.83	1.83	1 23	1.83	2.75M		0.000	0.404		0 497	0.324	0.409	2./5M	7 1 2	0.38	0.51	0.42	;	0.59	0.51	11,011	2.75M
ъ	U	2.5M		1.66	1.66	1.66	1.66	1.66	2.5M		0.488	0.367	704.0	0 453	0.295	0.372	2.5M		0.35	0.47	0.38	) )	0.54	0.46	IAIC.7	2 5M
4.5	4.5	2.25M		1.5	1.5	1.5	1.5	1.5	2.25M		0.44	0.33	0.41	0 !	0.27	0.34	2.25M		0.31	0.42	0.34	1	0.48	0.41	IAIC7:7	) JENA
4	4	2.0M		1.32	1.32	1.32	1.32	1.32	2.0M		0.390	0.293	0.361	) (i	0 235	0.297	2.0M		0.28	0.37	0.30		0.43	0.37	2.0IVI	2
3.5	3.5	1.75M		1.16	1.16	1.16	1.16	1.16	1.75M		0.341	0.257	0.316	0.206	906	0.260	1.75M		0.24	0.33	0.27	0	0 38	0.32	1.75M	
ω	ω	1.5M		ш	1	Ц	1	Ы	1.5M		0.29	0.22	0.27	O.18	5 7	0.22	1.5M		0.21	0.28	0.23	25.0	) သ	0.28	1.5M	
1	•			0.83	0.83	0.83	0.83	0.83	1.25M		0.243	0.183	0.225	0.14/	0 1	0.186	1.25M		0.17	0.23	0.19	0.27	0 77	0.23	1.25M	
S	Ł			0.66	0.66	0.66	0.66	0.66	1.0M		0.195	0.147	0.181	0.118	) (;	0 149	1.0M		0.14	0.19	0.15	0.22	) }	0.18	1.0M	
-				0.5	0.5	0.5	0.5	0.5	0.75M		0.15	0.11	0.14	0.09	!	0 11	0.75M		0.10	0.14	0.11	0.16	)	0.14	0.75M	
				0.33	0.33	0.33	0.33	0.33	0.5M		0.098	0.074	0.091	0.059	0.073	0 025	0.5M		0.07	0.09	0.08	0.11		0.09	0.5M	
12	3									L					-			L								

CD107a

INF314 ND050

6 6

5.5 5.5

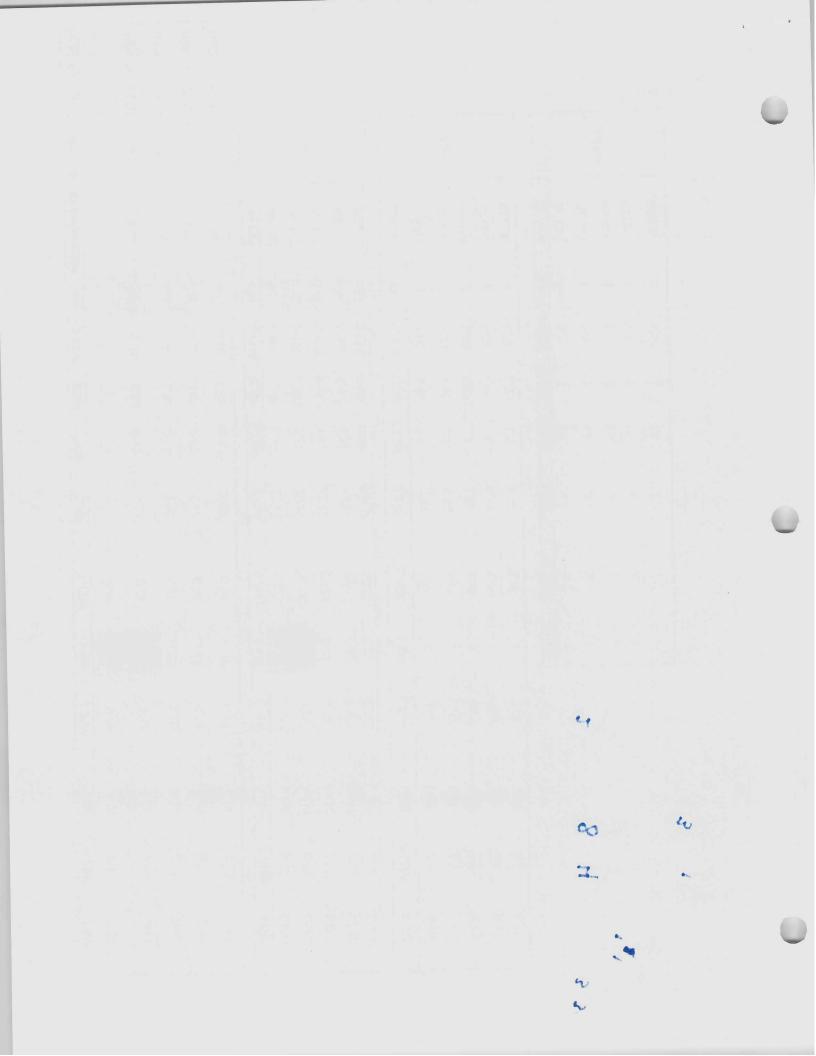
4.5 4.5 4.5

ND006









March 21th, 2023

Simplified Protocol

Whe end rocker CXCR3 the end

Collect, count, aliquot cells 2:3.0E+6 Cells R10 / 5mi polystyrene tube Bring volume uplo 1 mi R10, add 2 ul PMACCrt and CD107a Cap and incubate at 37°C for 6 hours Thaw cells, DNAse, count.

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

Add HotStain mix, incubate @37C for 30 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)

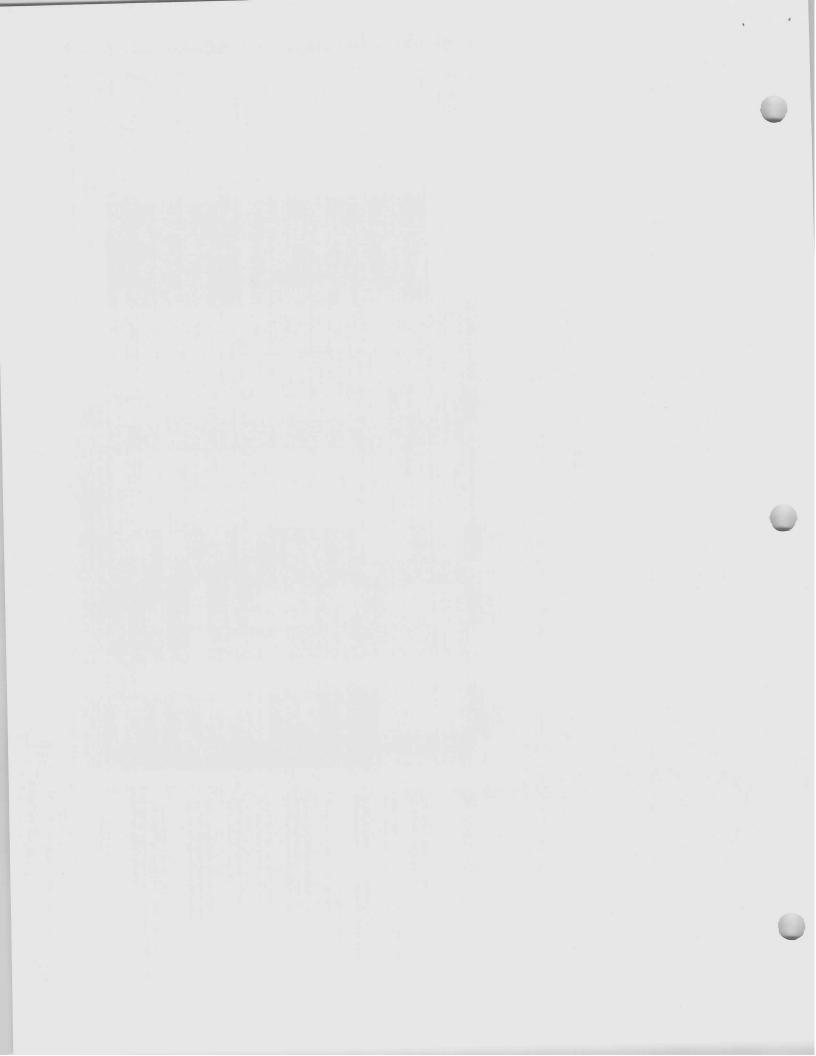
First PermWash: Second Perm Wash:

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 150 2 ml PermWash 1500 rpm 6 min

Cap tubes, wrap rack in foll, store at 4°C Resuspend in 70 ul 0.4% PFA.PBS

1.5/1.2 antibody



ILT & NK SFC Panel

																		-	100
	750 760						598	582	543	525	514	508	473	458	443	428	388	373	Spectrum
UV15 UV16	UV14	0	UV12	-	100	240	0		; S		UV7		1 1 1 1	UV5	UV4	UV3	UV2	LV1	
BUV805	BUV737			0	BIIV661	BINGLE	00000	BIIVECO		100	BIIV496		AF				<b>BUV395</b>		V
3	3			2	<u>.</u>	}	<u></u>	3		Ξ	3						[2]	-	
CD4	CXCR3			VUZ	CCR4		CD69			CDO							CD62L		
V15 V16	V14	V13	V12	<1 1	V10	<b>%</b>	- 8	5	6		\ <u>{</u> 5	4	<b>S</b>	ప	<b>S</b>	<u> </u>		+	
BV786	BV750	BV711		BV650	BV605		BV570	BV510			BV480			PacBlue	i	BV421			Violet
<u> </u>	[2.5]	4		[3.5]	<u> </u>			[1.5]			ធ		3	3	3	4		1	1
CCR6	IFNg	CD7		CCR7	CD56			CD45RA			CD161	e <sup>je</sup>	00 14/10	CD14/40	00.14	CD197			
B12 B13 B14	<u> </u>	B10	B8	В7	B6	B5	B4	Вз	B2		В1							t	1
Pe-Vio770		,	PerCP-Cy5.5	)	PE-CF594		PE	SparkBlue 550	FITC/AF488									טומפ	Rino
[3]			[4.5]		4		4	3	[1.5]	-,								1	
PD1			CD25 TNFa		CD26		NKG2D	CD3	Va24/hCD1d										
R R 7	R5	R. 7	R3 R2	R1															1
Zombie NIR APC-Fire 750		APC-R700	AF647	APC														Red	3/27/2023
3 22 12		<u></u>	[3.5]	[3.5]															٤
CD27		CD107a	Va7.2/hMR1	CD16															

3/21/2023

Unprixong schope

\* requires scapping mounty # Only NOOCE & Infill controls (-001070 PMA 03050)

group > ND050 otr/PM instanced per normal

Blip eDG9 experiment ()clot/clump) > (new fac/sie pop.)

A difficult to gak as brightest cose

need to drange cozs to PNA (Carready set)

A anne slorg us rare exits



