March 17th, 2023 2023 ILT 04 Specimen Status Location Conc Date **Tasks** Volume Ly Ly+Mon 2000011 Inf18Za-3 Total 3E+6 .3E+6HUN 1.5 10.5 7.03 (7,2) 9.63 1330-11 30/1 a-4 5955 1.5 (7) (7.8) 6.46 9.01 9.69 HEU-19 1030-11/ Inf281 Soyl 1.0 10.06 7.19 7.19 (A) (13.2) 9400/1 50/1 1.0 a-2 SLMG 5.71 5.71 8.83 6) (15,9) 1440411 HEU-hi Inf 191 (3) [7.5] 2.0 4.88 a-3 00 7.91 9.76 510011 Adalt NO050 1/13/23 HOP 15E6 7,5 Nom 6.47 8.66 9.7 [16] GOOLIV Adult ND006 15 E6 1/20/23 4411 1.5 5C'5 9:00 11.0 13.5 10:53am Than start Lym Conc Inf182° 20.19/3 1000 first and than 6.73 11.12 forst and sport Inf 281: 12,90/2 6.45 11:35 2nd spin done Inf191: 9.76/2 VIISHAM STAN 4.28 ide sheet setope 1:03 pm GM left 1:36 pm cells aliquoted 3m (14m) IM 3m (Incubation @ 2:22 pm > 8:22pm 3M 0 @ @ 1M 3m \$516 511 \$2000 \* NDOOE PMA got 107A added \$\$ snorthed one offs and added 1000 150 1.5 25 @ ZPIRM+ ZPI blecu... €511 @ 511 TBD effect. 3M @ 0 0 7.56 pm Ab prep starts ... Trax 1 1 15pine 8:31pm 0000 34 3M 3M 3M Unalique fed unstaineds 45011 +100 to the finder @ 8:43pm (1) 1 × 100 + 345/ (1) 4pe 20:49 pm (1) 3 × 100 + 221 H 50 me 2:09 pm 45×1+20/1 +100 31°C @ 9:26pm -> 9:56pm 70 N1 570pl +100

Tebe 10:5pm

10:30pm

Sec 10:5pm 2 10:38 pm Sphe 11:04pm

37 sec 1000 > 11:30 ND000 22 - PDI & cold)

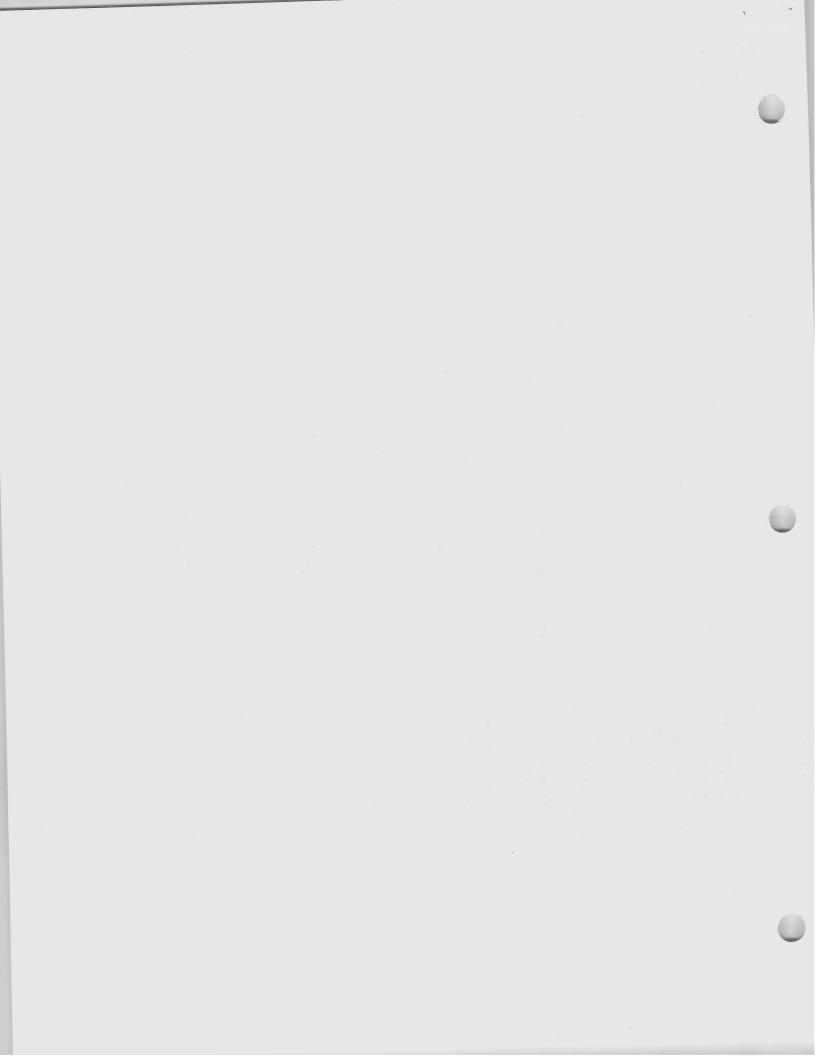
We se @ 11:26 - 711:56pm

// Samples He @ 11:18 pm -> 11:48 pm/

37°C Ses mark @ 11:31 Samples 42 -> 11:48 pm, 2.75 = 3 Sc1s 42 -> 11:56 pm, 7 Final FBS Spin @ 12:04 pm 2.75 = 3 Fixer @ 12:23 pm -> 33-> 43 pm 2 nd Pem Wash @ 1:10 am In frac @ 1:40 am (Sch away w/ 30 pl) -> 2:20 am Final wash @ 2:26 am Done @ 2:41 am

Split COMS RAN COLY
after COG9 mo colo
ansinal rea

NO APC CD3 ...



		Sample INF182 INF281 INF191 ND050 ND006
		Total 20.19 12.9 9.76 9.7 13.5
CD107a	R10	Volume  3 2 2 2 1.5
Sample INF182 INF281 INF291 ND050 ND006	Sample INF182 INF281 INF91 ND050 ND006  Sample INF182 INF281 INF182 INF281 INF191 ND050 ND006	Concentration 6.73 6.45 4.88 6.47 9.00
6 6	3M 0.55 0.53 0.53 0.54 0.54 0.67 3M 2 2 2 2	3M 0.45 0.47 0.61 0.46 0.33
2.75M 5.5 5.5 5.5 5.5 5.5	2.75M 0.507 0.490 0.352 0.491 0.610 2.75M 1.83 1.83 1.83 1.83	2.75M 0.41 0.43 0.56 0.43 0.31
2.5M 5 5 5 5	2.5M 0.462 0.445 0.321 0.446 0.555 2.5M 1.66 1.66 1.66 1.66	2.5M 0.37 0.39 0.51 0.39
2.25M 4.5 4.5 4.5 4.5 4.5 4.5	2.25M 0.42 0.40 0.29 0.40 0.50 0.50 1.5 1.5 1.5	2.25M 0.33 0.35 0.46 0.35 0.25
2.0M 4 4 4 4	2.0M 0.369 0.356 0.256 0.357 0.444 2.0M 1.32 1.32 1.32 1.32	2.0M 0.30 0.31 0.41 0.31
1.75M 3.5 3.5 3.5 3.5 3.5	1.75M 0.323 0.312 0.224 0.312 0.312 0.389 1.75M 1.16 1.16 1.16 1.16 1.16	1.75M 0.26 0.27 0.36 0.27
1.5 <sub>N</sub>	0.17  1.5M  0.28  0.27  0.19  0.27  0.33  1.5M  1  1  1	1.5M 0.22 0.23 0.31 0.23
1,25mm	1.25M  0.230  0.222  0.160  0.223  0.277  1.25M  0.83  0.83  0.83  0.83  0.83	1.25M 0.19 0.19 0.26 0.19
A CONTRACTOR OF THE PARTY OF TH	0.11  1.0M  0.184  0.128  0.128  0.178  0.222  1.0M  0.66  0.66  0.66  0.66  0.66  0.66	1.0M 0.15 0.16 0.20 0.15
11.5 S S S S S S S S S S S S S S S S S S S	0.75M 0.75M 0.14 0.13 0.10 0.13 0.17 0.75M 0.5 0.5 0.5	0.75M 0.11 0.12 0.15 0.15
D.S.M.	0.06  0.5M  0.093 0.089 0.065 0.090 0.111  0.5M  0.33 0.33 0.33 0.33 0.33	0.5M 0.07 0.08 0.08



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During Ind   Main   Telumerad   Holistan   India   Condistant   India   I					202		66	ume /sample	Pippette draw volume /sample	70	19.5	/sample	ppcc ulaw volulle / sample					
Deciding better   Deciding   De	700	9019		700	NEES.	700		oram	Dimidit	202	55		nette drawnalina	Pin				
	170				-				Brilliant	203	5.55		Media	R10		ONTROLS !!!	STAINED CO	And UN
	126			175.0		366		Total	Antibody	84	6.0		tibody Total	Ant				
						2.4							НП2	CD38	AFC/FIRE 810			
						78							0323	CD27	APC/Fire 750		RS	29
Propertical   Ref and   Primary									1:2500>				N/A	1/0	YIN BIGMO?		R7	28
Procedury   Refact   Procedury   Refact   Procedury   Refact   R										84	6.0		H4A3	CD10/a	ALC:NOO		R6	27
				1.0	9.5								3C10	THMU17-1PA	ABC BZOO		R4	26
	1.0		2000	9 9	0.7								200	Var officers	AlexaFiror647		R2	25
	70			21.0	1.5								308	CD16	APC		R1	24
Prior   Prio	35		The state of the s				2000						PD1.3.1.3	PD1	PE-vio770		813	2
Fiber Single-control() Ref ctrit   Pulmanageria   Risoratornom   Marker   Clone   Cl	7.0					16.8							MAb11	TNFa	PerCP-Cy5.5		oTo	:   :
Files   Single color (u)   Ref clark   Channe						16.8							M-A251	CD25	PE-Cy5		200	3 :
File	7.0					17							M-A261	CD26	PE-CF594		00	ا بو
File   Single color (u)   Red crit   Colore	7.0			16.8	7.2	2							1011	NKG2D	PE		B6	20
File   Single color(iii)   Refert   Colore   C													SK7	CD3	Spark blue 550		B4	19
Filter Single color(u) Ref ctr    Name   Clone   Marker   Clone   Constant   Clone   Clone						17	1.0						6B11	Va24/hCD1d	HIC/AF488		B3	18
File   Single color(u)   Ref car   Comming Car   Fluorochrome   Marker   Clone   Color   Col	21.0					21	1,7						11A9	CCR6	8V/86		B2	17
Filet   Single color (u)   Ref crit   Danne   Marker   Clone   Marker   Clone   Clon													827	IFWY	200		٧15	16
Filter Single color (u)   Ref drif   Conne   Marker   Conne   Conne   Marker   Colone   Col	14	1				14							M-T701	(0)	BV750		V14	15
Filter Single color (u)   Ref drift   Immaria (c)   Ref drift   Immaria (c)   Immari						14	Ď						G043H7	CCK/	BVZ11			14
Priest   Single color (u)   Ref cut   Duninavig Cut   Ref						14	1.0						TTHT.C	Occus	BVGSO			13
Priest   Single color (u)   Ref curl   Durincut   Durincut   Ref curl   Durincut   Ref curl   Durincut   Durincut   Ref curl   Durincut   Ref curl   Durincut   Durincut   Ref curl   Durincut   Dur	1												1	Chec	BV605			12
Priest   Single color (u)   Ref curl   Duninary (and part)   Ref curl   Ref Clyse, Spiked 30 min (and part)   Ref curl   Ref cur	1			9.8	0.7										The same of		1	
Priest   Single color (u)   Ref curl   Duman's Crit   Ref curl   Ref curl   Duman's Crit   Ref curl   Ref curl   Duman's Crit   Ref curl						28	2						H100	CD45RA	BV510			1:
Priest   Single color (u)   Ref ctrl   Durinchigent   Ref ctrl				28.0	2.0								HP-3G10	CD161	BV480		1	:   5
Price   Pric				28.0	2.0								SJ25C1	CD19	Pacific Blue		100	
Priest   Single color (u)   Ref ctrl   Unimary Etrl   Europericone   Marker   Clone   Vial Lot   Single   14   Marker   Clone   Vial Lot   Single   14   Marker   Marker   Clone   Vial Lot   Single   14   Marker   Mark						21	1.5						MSE2	CD14	Pacific Blue			
Priest   Single color (u)   Ref ctrl   Durnochrome   Nature   Clone   Validation   Colone   Validation   Striniti   Colone   Validation   Colone   Striniti   Colone   Colon	7.	0.5				21	T.5						A019D5	CD127	BV421			.
Priest   Single color (ul)   Ref ctrl   Diminum Stril   Fluorochrome   Marker   Clone   Vial Lots   Strill   Marker   Clone   Vial Lots   Strill   Marker				7.87	1.3								SK3	CD4	BUV805			
Priest   Single color (u)   Ref ctrl   Dimord/Figer   Fluorochrone   Marker   Clone   Vial Lots   Diming   14   (RT)   Mining   Mining   14   Mining   Min	7.0	0.5		9.8	1.0								1C6/CXCR3	CXCR3	BUV737		1	
Price   Single color (u)   Ref cut   Dumang Crit   Fluorochrone   Marker   Clone   Vial Lot   Strini   14					01	0.1	2.0						86	V82	T99ANR			.
Filter   Single color (u)   Ref cut   Unitading cut   Filter Colors   Marker   Clore   Vial Lot # stimility   14   (RT)   Min @RT   30mm @370   14   20mm @400   14   Hen   Min @400   Mi	14.0	1		1.0	0.0	20	20						1G1	CCK4	CTOACG			5
Filter   Single color (u)   Ref ctrl   During (tr)   Fluorochrome   Marker   Clone   Vallot #   During   14   (RT)     Min @RT   Somin@37C   14   Somin@4C   14   Shim   RBC Lyse, Shim   14   Shim   RBC Lyse, Shim   15   Shim   RBC Lyse, Shim   15   Shim   RBC Lyse, Shim   16				70	0.5								HNSO	0000	BINGSE	0		4
Filter Single-color(u) Referri Unmarigerri Fluorochrome Marker Clone Vallot stimili 14 (VD.15 min Tetramer 40 Hotstain In Tetramer 40 Hotstain Min @37C 14 30min @37C 14 30min @47C 14 Spiked 30 min @87T 14 1.2 16.8 Fix/Perm PRT 1.2 In Tetramer 40 Hotstain				8.0	0.7								KPA-18	CDG	RIIVS63			۱
Filter Single color (u) Ref ctrl Purmong ctrl Fluor ochrome Marker Clone Vallot # During 14 (VD 15 min Tetramer 40 HotStain min @RT 30min @37C 14 30min @4C 14 Hotel Hon Wallot # Hon Wallot # Spiked 30 min @RT 14 Honem Wallot # Hon Wallot # Hon Wallot # Hon Wallot # Spiked 30 min @RT 14 12 16.8 Filter # 1.2 16.8	1													Che Che	BUV496	7		
Filter Single color (u) Ref ctrl Oriminary Ctrl Fluorochrome Marker Clone Vial Lot # Stim!!! 14 (RT) min @RT 30min @37C 14 ColdStain then then WRT UV2 8U/335 CD62! CK11 K11 Clone Vial Lot # Stim!!! 14 (RT) min @RT 30min @4C 14 TkuPerm @RT BU/335 CD62! CK11				16.8	1.2									AF-UV6	AF			I
Filter Single color (u) Ref ctrl Orimonia (RT) Fluorochrome Marker Clone Vallot# stimil! 14 (RT) min (QRT 30min (Q37C) 14 30min (Q37C) 15 (ColdStain then then then then then then then the		(e x -			(				Month will store of		Section Section		SK11	CD62L	BUV395	2		1.
	200	Spiked 30 min		14	ColdStain 30min @4C		HotStain 30min @37C	Tetramer 40 min @ RT	L/D 15 min (RT)	-		Vial Lot#	Clone	Marker	Fluorochrome	er Single color (ul)		Ι.

## Simplified Protocol

Thaw cells, DNAss, count.

Collect, count, aliquot cells 2.3.0E+6 Cells R10 / 5mi polystyrene tube Bring volume upto 1 ml R10, add 2 ul PNAJCtrl and 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

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 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

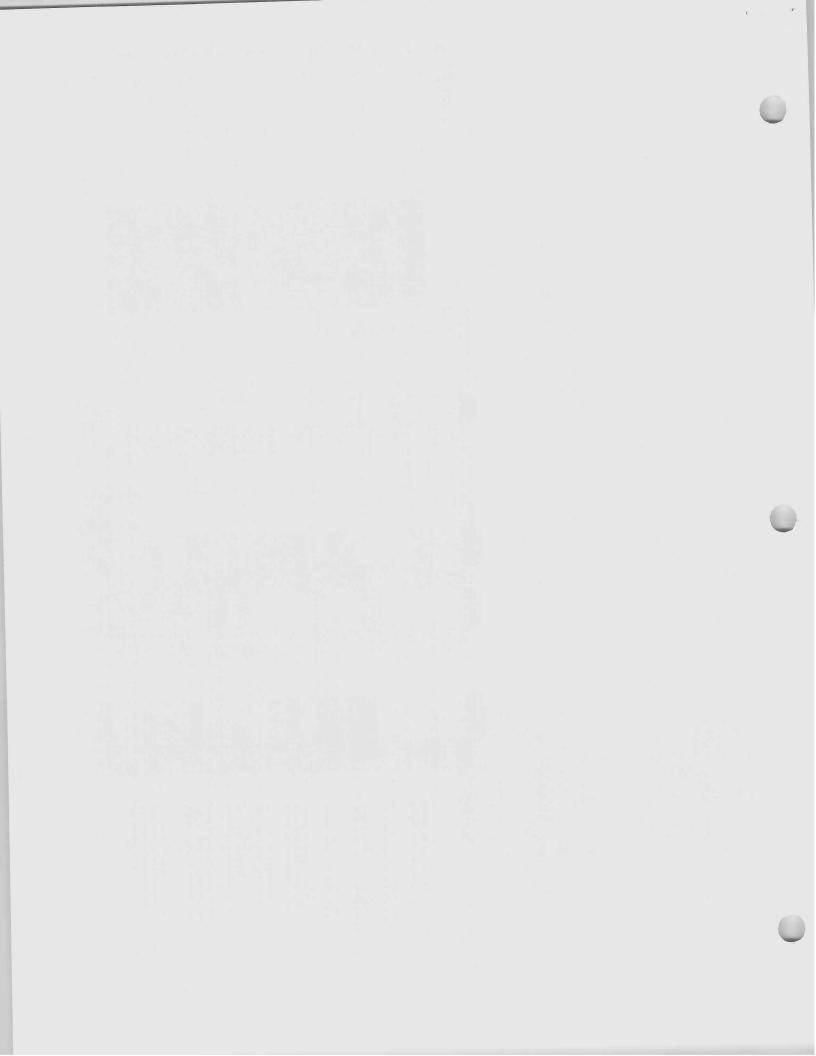
(vortex every 10 minutes)

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

1.5/1.2 antibody



ILT & NK SFC Panel

	812		760							598	582	542	525	514	508	473	458	443	428	388	373	opectrum
	UV16	UV15	UV14		UV13	UV12	11.00	010		(	1 P	UV8		UV7		DV6	UV5	UV4	UV3	UV2	LV1	F
	<b>BUV805</b>		BUV737				DUV667	BUV615		0	BIIV563			BUV496		AF				BUV395		V
Ī	3		[3]				<u> </u>	<u></u>		2	3			[2]						[2]	-	
	CD4		CXCR3				Vd2	CCR4	)	690				CD8						CD62L		
	V16	V15	V14	ζ.	V12		V11	V10	<b>6</b>	8	5	6	;		Si Si	<b>\$</b> 2	ప్ :	S :	<u> </u>		+	
		BV786	BV750	11.7AG	DV744		BV650	BV605		BV570	BV510				RV480		PacRlue		RV421			Violet
	3	<u> </u>	[2.5]	4			[3.5]	[3]			[1.5]			3	2	3	3	3	3		†	٦
		CCBs	IFNg	CD7	)		CCR7	CD56			CD45RA			0	0161	(F) (#)		77.00	2437			
814	α	B12	-	B10	В9	B8	В7	B6	B5	B4	ВЗ	B2		83							t	1
	Pe-VIO//U				PerCP-Cy5.5	PE-Cy5	,	PE-CF594		PE	SparkBlue 550	FITC/AF488									Dide	Diss
	<u> </u>				[2]	[4.5]		<u> </u>		4	Ξ	[1.5]									1	
	PD1				TNFa	CD25	010	CD26	ļ	NKG2D	CD3	Va24/hCD1d										
R8	R7	R <sub>6</sub>	R5	R <sub>4</sub>	R3	R2 -	<u> </u>															1
APC-Fire 810	APC-Fire 750	Zombie NIR		APC-R700		AF647	ABC														Red	3/17/2023
[3]	[2]	=		ය	2	<b>3</b> (3)	3															l iii
CD38	CD27	ĽÐ		CD107a	401.14.	Va7 2/hMB1																
															-						_	

March 17th, 2023

WDOOG the APRIAT experiment under which cood ~ 20ndie. 2023\_ILT\_04

Initial Unmixing: No Cords ... excess Inf182

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