	Specimen	Status	Location	Conc	Date	Notes		2023_AB	_TNFa	
36	2009 8375	Infant COPBMC	as in a		Date	Notes	Volume	Lym	Lym+Mon	Total
9.	CQ1106			×10.687			0.5	8.97	16.60	
′ - F	30098640	cord		10.97	6/2/14		2	0 22		
oe.	NOOSO	Adult 8	-	7,556	6/5/23	TT	-	8.33	12.10	16.66
	C. 6022	Adulta		6.847			1	14.30	19.10	
r.		760.10		7.554	3/1/3		0.5	10.20	12.50	
						-	0. 3	10.20	12.30	
-	,									
						4 .11.			\	
						Jac pt	7 - 1	gra Del		
-										
1										
	Thew		E		1 . 1					3M
	DNAse		Γο	rmulo	dehyd o	smell from	000	Eord	00 %	300
	count stain in line exce					e son III			00	2.25M
	41 alignot			(	11 C	PMASE'S		Nigena	00	2.25M
3:	11pm Inc	chat.u-	start	7	DICK SC	50 11:06 pm >	0/0 .	N Doso	0.00rd	215440
_	79:11	pm		5	97/-	36pm		_	0.001d	6.23M
7:5	17 Ab pre	070	2:20		+ / < 6	old @11:43pm-	>			220
81	47om R	eas - 1	· 30						FED 5	5() 1.32
	47pm R	-gen!	s prepped		00:1	Perm @ 11:50 → 8 0 → 00:20	)0.00 - HA	2 <2	<1.5	
	Piledo	9:53	Pu >	Rb	xc /45	E @ 00/15-18	31	2/1	7.5 7.	5
1	1-10e 10	11:			4/	200,32->47	spin	1storash c	21:37 am - @ 1:49 am -	
	Sc Spina	9 10:24	pm		2.1	-0,32-147	In	and a agh	es.49an-	> 1.54p
11	nd settlo.	38pm	g. <b>1</b>	* 1. 1	Sind	50 wosh@0014	/		/ Comment > 6	W 1 8 2 2 2 2 2
610	Sel Hots o	10:44	pm -> 10	1. 14pm	Int	pletic Penyeng	1.02-7	1272	2-32 W	one 21
W	5pl Hot's @	(0.00)	@11:30	co pm	4111	blumsple 1:	-> 1:50c	ran	/	-

Old ND050 ... couldn't recall of COZE had been added to hat (unlikely, only recall broken) so added 0.5 pl Pecf594 eDEG e inhae 12.5 -> 1.5 PerCP-Cy5.5 ID26 (suspect works) (dude...) TNFA PerCPG5.5 -> 2.5 pl Juil see how eater 40 mindes TNFA Pedazzlo74-> 1.2 pl -> ortarand 2:43pm resus in 25pl

Done @ 324 am

2023\_AB\_TNFa FSC 140 sec 380 33 c-B 397 Sc stort @ 4:43pm: EHLA-DR actuation? Scs @ 5400 ents/120 50 ledica FSC I need to odyst ssc-B in the fitter next ding? Lod ferrer doblets of this processing. FORP3, VOZ & VAZZ areNDO50 unstaineds ... 11 Sc CDIGI Stamed/ HiA - DR fine Cord Se done c 5:33 pm: 25011 se dosc CO POMO CQ 1106 C6022 NDOSO 16K/1.2K 11.41 1536 1211/.71 1211/.71 60M 19M 48/1 1.1 M 45/1 IM MIL PARION Smilehal?

chilold at chlastared.

Done @ 6:25pm

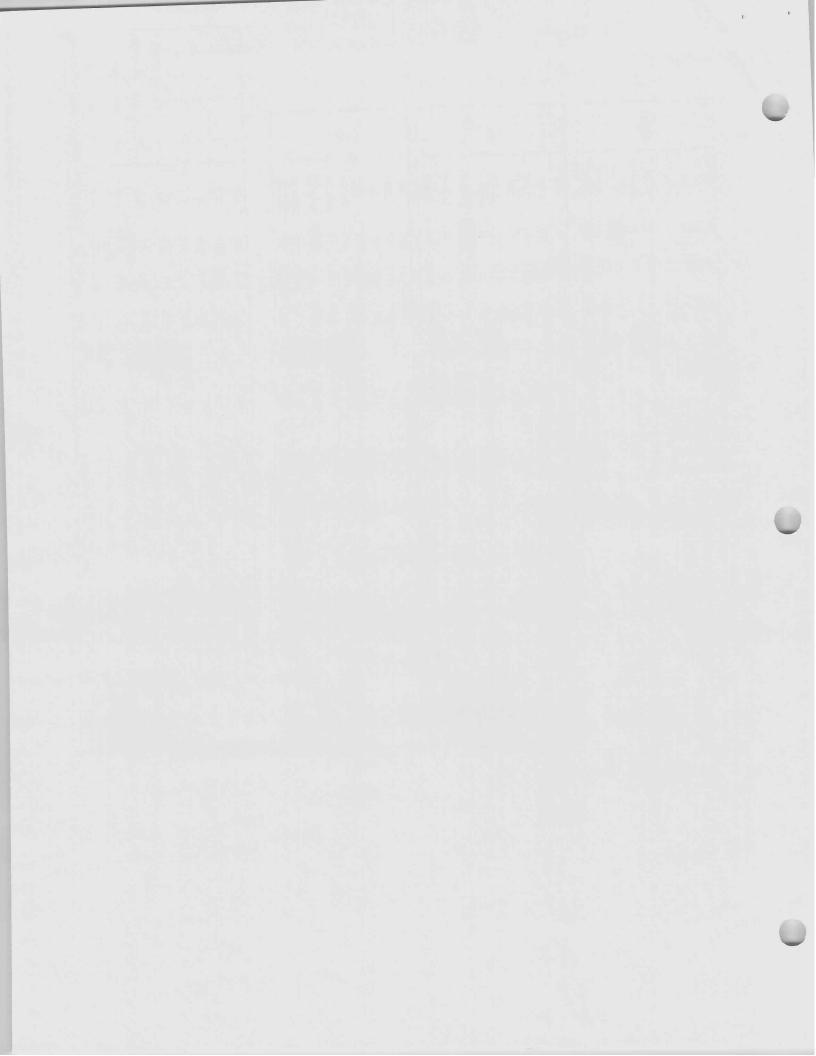
Initial unix 7/12/23 @ 7:48pm

Bed 27 June 2.48 80

2:55

						CD10/a										į	PMA										R10				
NO	2	ND	N <sub>F</sub>		Z T	ND050	CVD 6022	INF 8640	INIT OCAD	Sample INF 8375		ND	ND	N	IN F	ND050	CAD 9077	CVD C022	INIT OS AS	INIE 027E	Sample		N D	ND D	N <sub>F</sub>	N N	ND050	CVD 6022	INF 8640	INF 8375	Sample
6	) (	0	6	c	ת	6	6	6		3M		2	2	2	2	2	~	2	2	, J	Σ	1.00	-2 00	-2.00	-2.00	-2.00	0.79	0.70	0.64	0.67	3M
5.5	, ,	7	5.5	0.0	ח	5.5	5.5	5.5	0.0	2.75M		1.83	1.83	1.83	1.83	1.83	1.83	1.83	1.83	Z./5IVI	3 7500					-1.834			0 586	0.609	2.75M
л	U	л	G	U	,	Ŋ	σ	5	v	2.5M		1.66	1.66	1.66	1.66	1.66	1.66	1.66	1.66	2.5M		/00/1-	1 667	1 667	-1 667	-1 667					2.5M
2	4.5	n -	4.5	4.5	The State of the S	4.5	4.5	4.5	4.5	2.25M	ŀ	ν 7	1.5	1.5	1.5	1.5	1.5	1.5	1.5	2.25M				1.50						1000	2.25M
>	4		4	4		4	4	4	4	2.0M	7.32	1 2 2	1 3 2	1.32	1.32	1.32	1.32	1.32	1.32	2.0M		-1.334	-1.334	-1.334	-1.334	0.526	0.466	0.426	0.443	2.014	2 OM
i	ω. <b>5</b>		л	3.5	į	υ (	υ π	3.5	3.5	1.75M	1.16	1.10	1 16	1 16	1.16	1.16	1.16	1.16	1.16	1.75M		-1.167	-1.167	-1.167	-1.167	0.461	0.408	0.373	0.388	T./5 V	4 7774
(	ω	U	ن —	ω	C	υ (	υ —	ω	ω	1.5M	L		, <sup>–</sup>	ہ د	<u>→</u> 1	<u>н</u> ,	_	ы	1	1.5M		-1.00	-1.00	-1.00	-1.00	0.40	0.35	0.32	0.33	1.5M	
					*						0.83	0.83	0.83	0.63	0 83	0 83	0 83	0.83	0.83	1.25M		-0.834	-0.834	-0.834	-0.834	0.329	0.291	0.266	0.276	1.25M	
						0.5	) 1	>	Volume		0.66	0.66	0.66	0.00	0.00	0.66	0 66	0.66	0.66	1.0M		-0.667	-0.667	-0.667	-0.667	0.263	0.233	0.213	0.221	1.0M	
						70.2	3 6	8 22	Lym		0.5	0.5	0.5	) (. )	o .c.	0 0	0	0.5	0.5	0.75M		-0.50	-0.50	-0.50	-0.50	0.20	0.18	0.16	0.17	0.75M	
					21.76	5.1	1 0:00	16 66	Total		0.33	0.33	0.33	0.33	0.33	0.55	0 1	0.33	0.33	0.5M		-0.333	-0.333	-0.333	-0.333	0.132	0.117	0.107	0.111	0.5M	
						10.88			Average												L										

Sample	Total	Volume	Concentration	AS	3 7EM									
INF 8375	4 48	ם		O IA	IAIC 7.7	2.5IVI	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M 0.5M	0.5M
	i	į	0.90	0.33	0.31	0.28	0.25	0.22	0.20	0.17	0.14	0 11	90.0	0.06
INF 8640	16.66	2	× ک ک	0 30	2									0.00
	!		0.2	0.30	0.33	0.30	0.27	0.24	0.21	0.18	0.15	0.12	0.09	0.06
CAD 9055	5.01	0.5	10.02	0.30	0.27	75.0	0 22	3	•					0.00
ND050	14 3			)		i		0.2.0	71.0	0.15	0.12	0.10	0.07	0.05
		1	17:50	0.21	6T.0	0.17	0.16	0.14	0.12	0.10	0 09	0.07	0 00	3
NF	2	2	1.00	3.00	2 75	о ЛО	ر د 1	)			0	0.0	0.00	0.03
Z	ى	)				0.77	22.2	2.00	1.75	1.50	1.25	1.00	0.75	0.50
	1	٨	1.00	3.00	2.75	2.50	2.25	2.00	1 75	7	)			
ND	2	2	100	8	1 1					1.50	C2.1	1.00	0./5	0.50
<b>?</b>				0.00	2.75	2.50	2.25	2.00	1.75	1.50	1 25	3	0.75	0
20	2	2	1.00	3.00	2.75	2.50	2.25	200	1 75	3	<b>;</b> ;	1.00		0.50
								1:00	1./3	1.50	1.25	1.00	0.75	0.50



		And UNSTAINED CONTROLS !!!			29 R8	28 R7	27 R6	26 R4	25 R2		23 YG9	22 89	<b>21</b> YGS	20 YG3	19 YG1	<b>18</b> B3	17 82	16 VIS			1	12 V10		11 47		10 VS	V3	9 V3	8 V1	7 UV16	6 U14	5 UV11	4 UV10	3 UV9	2 UV7		1 UV2
				APC/Fire 810	APC/Fire 750	Zombie NIR	APC-R700	Sicke Fluor 647	Alexa Elica 617	ARC	BE-vicazo	ParCP-CVS	BE C.F	DE Davideo	Spain blue 550	Smark bling the	Alexa Elior 189	BV786	BV750	BV711	87650	BVS05	87/270	BV510	BV480	racilic bine	Pacific blue	Badia Bl	BUV805	BUV/3/	BUV661	STOVOG	BUV/615	BUV496	BINGOO	AE	currype name Fluorochrome
				CD38	CD27	L/D	CD107a	11.2	CD39	HLA-DR	CD26	CDZS	PANI	PD-1	CD3			COBC	IENa IENa	VANO	CAUKS	CVCD	CDTONA	CDASEA	CD161 -Bio	6019	CD14	CD127	CD4	CXCR3	Vd2	CCR4	CD69	CD8	AF-UV6		
- Francisco volume /sample	Pinnette	R10 Media	Antibody Total	(нп2)	(0323)		(H4A3)	(MQ1-	(A1)	(L243)	(M-A261)	(M-A251)	(MAB11)	(EH12.2H7	(SK7)	(236A/E7)	(HAS)	(027)	(SCIU)	(G043H/)	(J252D4)		(HILLOO)	(History)	CD161 -Biotin (REA631)	(HIB19)	(M5E2)	(A019D5)	(SK3)	(1C6/CXCR3)		(161)	(FN50)	(RPA-T8)		(DREG-56)	
19.5	14.5	1 00	60				6.0									_		-	-				-	_					-	_		_					
-	Brilli	Aiille			1		60																														
/sample	Brilliant Stain	Antibody Iotal			71.60007	2500																		1.5										-			and minca
63.5	50	16.5	1.6	1			T	4.4	1 2		4.6	1 2	<1.2>		1		1.5			-	2.5						410	1.0	1	1	0.7	1 25	1	1	+		@37C
	500	165	16	20				11	13		7.7	17					15			10	25	1					-	15			63	-	1				C
61.7	50	14.7							0.0	00			1.4	1.2					12		1	0.7	0.7	200	2.0	2.0	2		26	0.7	0 1	0.0	0.7	0 1	1.2	2	30min @4C
	500	147.0							0	0			7.7	12	3				10			,	7		02	30	3	•	25	1	,	U	,		12	3	4C 10
																								Strep:			Ī		9	3							15 min
				_																				0.8								-	-		-		Fix/Perm
58.1	50	11.1				32	1.2	-		<2.5>				0.1	U		<u>7.2</u>		0.7	01								0.5		0.1		1		T			Stain 40 min
	500	111					12			#####	5	#####	G	1	50	-	17	1	,	,								5				10			ji.		10

0.8 9.6 10	4 7	3.84	
12	10 PBS	9.6	0.0
		12	NIN SIGNA

Number Samples	Total	FBS	RBC Lyse	Fix	Perm	PF A
Seiduper squipes	10	80		10		
Number Unstained		27		1	Ī	T
Minister C. F. C. Co.	0	32	2.4	8	32	
Morning Surface Scs (29)	27	27	,	2		-
Number Intracellular SCs		-		1	TOO	
The second of th	os	00	0	80	64	0.16
Total		147	5.4			
	The state of the s	2000		1		Ī
		132.3			255.6	
FBS 73.5		Perm	700			
The state of the s			4.07		rix	13.25
/3.5		Water	256		Diluent	39.75
-						
PFA 0.124		RBC	0.54	_		
PBS 1.116		Water	786			

## Simplified Protocol

Thav cells, DNAse, count.

\*Resuspend at 156mid RR.) rest cells for <12-hrs in a 6-well plats, >
Collect, count, aliquoir cells 2.0E+6 Cells fini polystyrene tube
Add antigens, stim antibodies, and DuringStim antibodies in \$60 ul R10
Cap and incubate at 37°C for 2 two hours Add golgi block, and continue to incubate for <6> hours

Add CD161 biotin antibody for 10 minutes at RT Add to it HotStain mix, incubate @37C for 30 min Wash 2 ml 5% PBS.FBS 1400 rpm, 6min

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 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

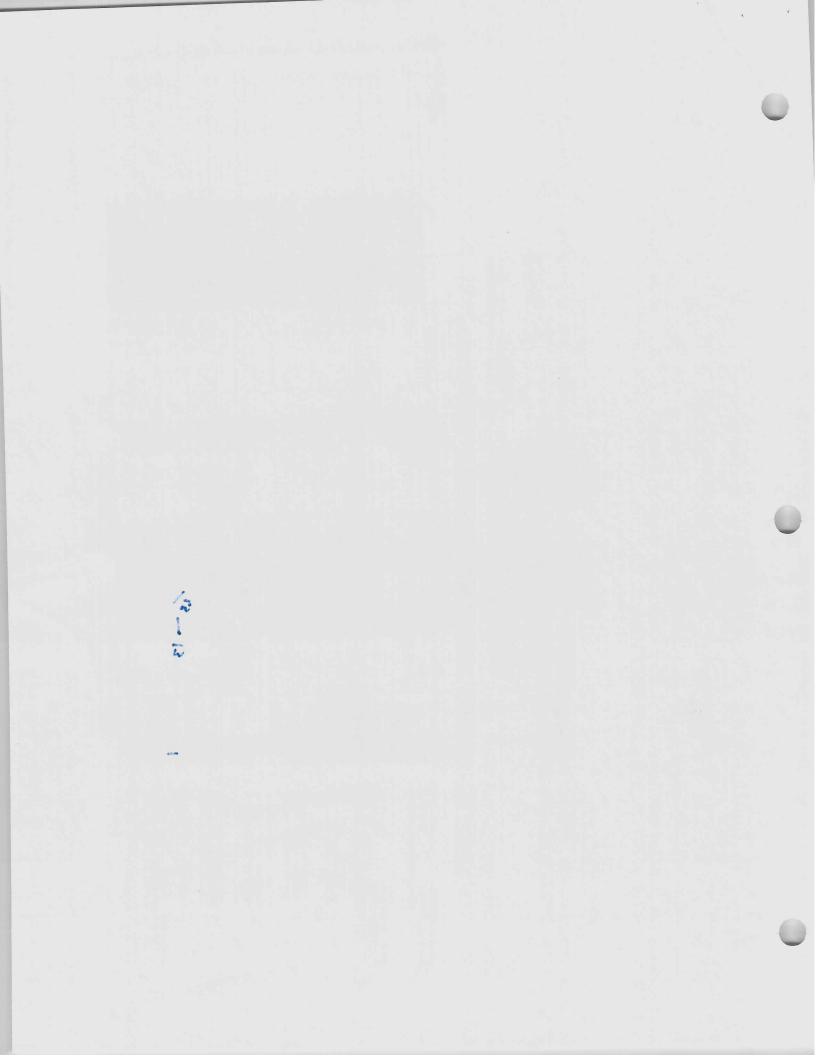
Add Streptavidin Mix, incubate @ 4C for 15min Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

First PermWash: Second Perm Wash: 1 ml Nuclear FixPerm, incubate @ 4C for 30min (vortex every 10 minutes)
2 ml NuclearWash 1500 rpm 6 min
2 ml NuclearWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min First PermWash: 2 ml Nuclear Second Perm Wash: 2 ml Nuclear 2 ml NuclearWash 1500 rpm 6 min 2 ml NuclearWash 1500 rpm 6 min

Resuspend in 100 ul 0.4% PFA-PBS

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



514 UV7 BUV496 525 542 UV8 582 UV9 BUV563 598 613 UV10 BUV615 664 UV11 BUV661 679 UV12 717 UV13 750 UV14 BUV737	UV8 BUV496 UV8 BUV563 UV10 BUV615 UV11 BUV661	UV7 BUV496  UV8 BUV563  UV10 BUV615  UV11 BUV661	UV7 BUV496 UV8 BUV563 UV10 BUV615	UV7 BUV496 UV8 BUV563	DV/8 BUV496	DV7 BUV496	UV7 <b>BUV496</b>	7		443 UV4 458 UV5			373 UVI
CXCR3				CCR4 V82		CDse		CDa				EDE CD62L	
	V14 BV750	V13 <b>BV711</b>		V10 BV605 V11 BV650	V9 DV3/U	W BV510	V6	V5 BV480	V3 PacBlue		∨₁ BV421		Violet
B12 CCR6 B13	IFN <sub>γ</sub>	Wa7.2 B10		CXCR5		CD45RA		CD161	CD14/19		[45] CD127		
2	1	B9 PerCP-Cy5.5		B 86	B. B.	B3 SparkBlue 550		B1					Blue
		CD26/TNFα				FoxP3 CD3							
Pe-Vio770			YG5 PE-Cy5	YG3 PE-Dazzle594	YG1 PE								Yellow-Green
HLA-DR			CD25	TNFα/CD26	[50] PD1								
R6 Zombie NIR R7 APC-Fire 750		> >	R2 AF647										Red
Viability CD27		CD107a	CD39										

