									yo SFC F	anels #	1#2	
	Specimen	Status	Location	Conc	Data	TD. I	T = -	1	1:100			
7				Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	1.5E+6	0.5 E+6
	ND027	Abut		NE6	4/4/07		3 ml	1E6	1.73 1.63 E4	(3)		0.3 1210
1539H	ND027	Alelt		10E6	4/4/7		3 ml	.9E6	1:41 EG	2.7		
									,	7		
	0:47 cons	d .										
	0:47 cons	stain e	starts ads	1)	CEI	DIENT 350	0 (4/1) E				1 1200	medier

400 1:17pm -> 1:32pm

+ backbore

20 SC CD16 (4/1)

3050 CD27(1.5pl)

\$ 0 unstained SC RR low signal V

5 vials

Tetramer 1:44 : 2:25

\$ 6:40pm new wellsheet @ 6:53 pm Sample coming

He Sels@ 2:32pm -> 3:02pm 37'c added @ Z:37pm -> 3:07pm

3:03 = 3:06 RBC lyse other controls

3:20pm > 3:50pm \$4c stain -> 3:51pm RBclyse -> 3:54 3:21 pm Fix Rom &s -73:31 > 3:41 pm

Sels first perm wash

4:05 FIRPERM -> 4:15->4:25

4:48pm > 5:28pm Intracelle

5:35 pm/ Stains done

36120 - 17621,4 the state of the s The second of the second of

R Filt Single color 10 Act on Color Colo			68.6		55	Pippette draw volume /sample	Pippette draw		19.5	and accounted	/sample						
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1 10 10 10 10 10 10 10	78.4			22.4	5.6	dy Total	Antibo	27.2	6.8	Total	Antibody				CONTROLS	STAINED	And UN
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				tion to the same								CD28	APC-Fire750	<		00	21
				0	-	1	+					L/D	Zombie NIR			R7	
# Filter Single color (u) Red cttl Uname (Fluorochrome Marker Loope Valors)				,		100	*20	A	6.0		(H4A3	CD107a	APC-R700	7		R6	20
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	4	staining	4 Surface	11 19 1) for 37oC	etramer (RT	15 min	10000					Fluorochrome		ter Single color (ul) Re		1

Simplified Protocol

Aliquot cells 2.06-6 Cells/tube
Raise R.10 + cell volume to 500 ul
Add 500 ul of PMA-innomycin/Ctrl
Incubate 6 hours @ 37C
Wash with 2 ml PBS, 1400 rpm, 6min
800 ul Live/Dead @ RT for 15min
Wash 2 ml 5% PBS-FBS 1400 rpm, 6min
Add Tetramers, incubate @ RT for 40min
Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

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

Add cold Surface mix, incubate @ 4C for 30min
Add 300-500ul 1x RBC Lysis for 3 minutes
Wash 2 ml 5% PBS-FBS 1500 rpm, 6min
300 ul Oxfofit/perm, incubate @ 4C for 20min
Wash twice 1 ml PermWash
1500 rpm, 6min

Add Intra Staining, incubate @ RT for 40min Wash once w/ 2ml Perm Wash Resuspend 100 ul 0.4% PFA-PBS, store 4C

50's 16-74
2771.5?
RA-70.7

gd T cells - Cytotoxic

	750 U								514	508	473	458	443	428	388	373	Spectrum
UV15 BUV805	UV14 BUV737	UV13	UV12	DO VOO			UV9 BUV563	CV8 	UV7 BUV496		UV6	UV5	UV4 AF350	UV3	UV2 BUV395	UV1	VV
3	<u> </u>			[3]	[3]	3	<u>.</u>		[2]						5 2	-	
CD4	CD27?			Vd2		C	CDee		CD8				CD16?		CD62L		
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BV786	BV750	BV711		BV650	BV605		BV510			RV480	9000	DacBlua		BV421			Violet
[3]	[2.5]	[4]		[3.5]	<u> </u>		[1.5]		3	3	Ξ	=	127	E		\dagger	
CCR6		CD127?		CCR7	CD56		CD45RA) 	00.00	200	CD14/19		17100	CD4373			
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Pe-Vio770			PerCP-eflour700		PE-CF594	PE	SparkBlue 550	AF488								pine	Di
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Zombie NIR APC-Fire 750 APC-Fire 810		APC-R700	AF647	APC												Red	9/23/2022
<u> </u>		<u>ය</u>	[3.5]	[3.5]													22
L/D CD28 CD38		CD107a	hMR1	NKG2A													

9/23/2022

Unmixing WIS/16 bottomby out

