(reusing ref controls ween khalf ago)

	November	22nd ^h , 2	022										
Differ			-						Tetramer '	Titration	ıs		
1 =	Specimen	Status	Location	Conc	Date	Tasks	Volume	T					3
wit'	MOOR	Adult					volume	Ly	Ly+Mon	Total	3E+6	2E+6	C) Tables
44acjul				15E6	11-14-22	2	2	6.32	8.00	12.64	475/	316/1	47
440gpl	(2)	11		((()		2	5.13	6.56	10.26	585M	389/-1	52
resocity	(3)	11		le	l _t	more deadith	2	5.47	7.13	10.94	548H	366/1	54
4011	(4)	10		(ı	4		2	4.71	6.21	9.42	637H	425 pl	63
4041	(5)	11		10	cl	movedeodish	2	4.46	6.00	8.92	673H	4481	67
Man Ab	18ml (3-2: 372 abs 6	Pen wa Pen wa Pen wa (48)	pm @ 12: sh and and and and and and and an	9 .3 .7mls 1Bc ysc	6.4	and the state of t	Bol unline	ded houses h caded houses	$\frac{0}{3} = 2$ $\frac{3}{3} = 2$ $\frac{3}{3} = 2$ $\frac{3}{3} = 2$	2 / = 1	trained for ample some cell	ls recover rate	>)

In @ 6:13 for 4° = > (6:43 pm)

Ralps = @ 6:53 pm > 5pin @ 6:51 pm

FurPein@7:11pm > 25/531

First washer 7:34pm

Done @ 7:54pm



		And UNSTAINED CONTROLS !!!			R8	R7	16 R6		15 R2		813	B10	88	13 B6	84	12 83		CTA OT			9 V13		8 V10		7 V7	6 V5	V3		4 V1	3 UV16	U14		2 V11	ULYU	UV9	1 UV7		# Filter Single color (ul) Ref ctrl name UV2	
				Arc/rite 610	ADC/File Coo	APC/Fire 750	Zombie NIR	APC-R700	AlexaFluor647	APC	DE-min 770	PerCB-Cys s	DE-Cus	PF-CF50A	DE	Spark blue 550	AlexaFluor 488	BV786	BV750	BV711	BV650	BV605	BV570	BV510	BV480	Pacific Blue	Pacific Blue	BV421	BUV805	BUV/3/	PINCES	BUV661	BUV615	BUV563	BUV496	AF	CKCANG	P	
				CD38	CDZ/		CDIO79	CDIAN	PWID	PUI	TNFα	CD25	chize	NKGZD	5000		bont 4	Cons	IFN _V	CD7?	CCR7			CD45RA	CD161	CD19	CD14	CD127	CD4	CXCR3		V82	CCR4	CD69	CD8	AF-UV6	CD62L	Marker	
/sample	Pippette draw volume	R10 Media	Antibody Total	(нпт2)	(0323)		(H4A3)				(MAB11)	(M-A251)	(M-A261)		JSK/		(11A9)	Total Line	(R27)					(Hi100)	(REA631)	(нів19)	(M5E2)	(A019D5)	(SK3)	CXCR3)	(106)	(B6)	(161)	(FN50)	(RPA-T8)		(DREG-56)	Clone	
7.67		20.5	0.0																+	1	-	+	1				-		1		+		1					Vial Lot #	
		174	0				N N													-							100											During	
/sample	Pippette draw volume	Brilliant Stain	Antibody Total			<1:2500>		<1:500> (5)								<1:xx>(1.2)														3.2							100,000	8.5 L/D Tetramer	
52	50		2.7										1.2				1.5									-		+					-	+		-			Special and Special Sp
	425		23										10.2				5 12.8													(See							CHILD	37oC (RT) for	The second second
65.2	50.0		16.2						0.7				3	7:1	10		òo		1.0		1.0		0.7	2.0	2.0	2.0	1.5	1.5			0.7			0.7		1.2	4oC for 30min	8.5 Surface staining	
	425		137.7					0.0	60					T.0.7	300				8.5		8.5		6.0	17.0	17.0	17.0	12.8	12.8			6.0			6.0		10.2		8.5	
				-		-																															Perm	Lysing, then CytoFix/	
50	50	,	-											0.25										0.25				0.25						0.25			40min Intra mix	Lysing, then CytoFix/ Intra mixRT for	
	425	9	χ											2.13										2.13				2.13						2.13			/	8.5	

Simplified Protocol

Incubate 6 hours @37C Add 500 ul of PMA-ionomycin/Ctrl Aliquot cells 3.0E+6 Cells/tube Raise R10 + cell volume to 500 ul

Wash with 2 ml PBS, 1400rpm, 6min

Wash 2 ml 5% PBS-FBS 1400 rpm, 6min Wash 2 ml 5% PBS-FBS 1400 rpm, 6min Add Tetramers, incubate @RT for 40min 800 ul Live/Dead @RT for 15min

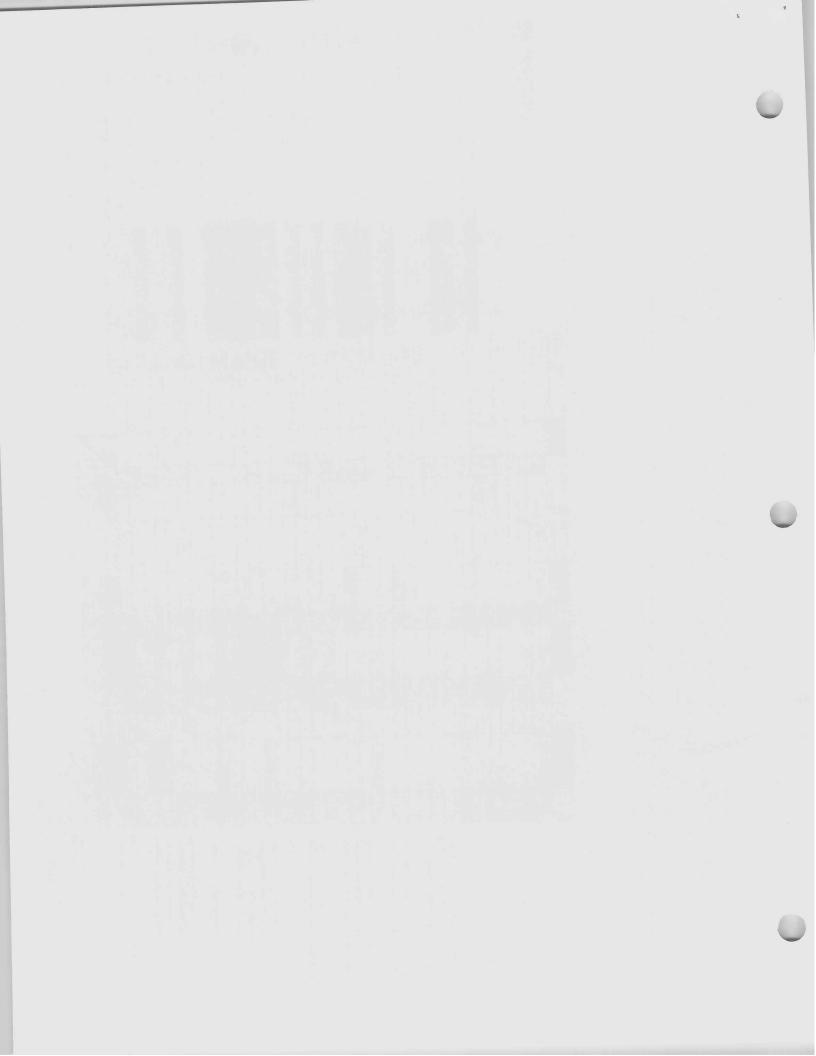
Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min Add hot Surface mix, incubate @37 C for 30 min

Wash twice 1 ml PermWash 1500rpm, 6min 300 ul Cytofix/perm, incubate @ 4C for 20min 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

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

2 ml Pernamosh 8 ml FBS-PBS

6



ILT SFC Panel - Titration Test

	812 UV16	783 UV15	760 UV14	738	717 UV13	697 UV12	679	664 UV11		598												obecumil
	BUV805	15	14 BUV737		13	12			UV10 BUV615		BUV563	UV8	- Company	UV7 BUV496	DOMESTICAL PROPERTY.	UV6 AF350	UV5	UV4	UV3	UV2 BUV395	UV1	۷۷
	m cov		[3] CXCR3						⊠ CCR4		[3] CD69			[2] CD8						[2] CD62L		
V16		V15 RV786	V14 BV750		V13 BV711	V12			V10 BV605	\\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\	V° BV570			V5 BV480			V3 Pac Rino		V1 BV421			Violet
TI TI	יין כראס	0000	[2.5] IFNg	- CU/	202		[22]				[1.5] CD45RA			[3] CD161		111 0014/18			[4] CD127			
B14	B13 Pe-Vio770		-	B10	B9 PerCP-Cy5.5		B7	B6 PE-CF594		PE	B3 SparkBlue 550	AF488		B1							biue	Dino
	। PD1				[2] TNFa	[4.5] CD25		[4] CD26		[4] NKG2D		[1.5] hCD1d										
	R7 APC-Fire 750	R6 Zombie NIR	R5	APC-R700	R3	AF647	R1 APC														Red	770717111
	[2] CD27	[] []		[3] CD107a		(and	[3.5] CD16															77

tetramer ditration our samples @12:13 pm

deflicated IIT final, deleted extra parameters

(Fixed morayles applit in the V2-V7 AF signal)

Unstained running - 10:300 Event rate (plated 3M cells)

Monocyte FSC settings word fine for this one; not so in Monocytes if lattingdeblets

(600)

Going to go ahead and our CD3 & constanted cell sets a unstand back of in this

Unmixing & Did it just crash & Sorta... (16 colors)

hMRI looks to be at lover deletar this go around (knowing issuem hads)

