Bloody Hell - Roy 21-22B

	Specimen		Location	Conc	Det		Bloody Hell – Box 21-22B										
824	Inf-1015-7	HU9	Box ZBB1	Cone	Date	- 1000	Volume	Lym	Lym+Mon	Total							
31,	4-5	VYXN	2			Lysed, but alive?!?!	1	3 (75)		Total							
12222	Infloca-5 a-5	HU8	Box 21B	17453		Perry happy	•	20176	3.89 Ef								
13100.	Inf1073-1	111) 7	7,2 (3)	1,437		,	- 1	12,30	16.3 EG	3ml							
40/1	4-5	VCH6	B.6 ()	000		he wild cryotial Ind red Blood cells I	,	-,,,,		Office							
1800,	Taf1122-6	HU F	Bruk 22 B.	6.667		Wested Stypevial		7.37	11.4 11.4 11.4 11.4	5,5ml							
40/1	u-5 Inf1122-6 a-4	VFAQ	I.6 (1)			red Blood cells !	1	0.26	12.9 EG	, ,							
						7 7	•	8.26	1201 24	6.5ml							
5	tait @ 1:56	pm 5	Dia @ 2-10	DALAS	2	2.0											

Start @ 1:56 pm Spin @ 2:10 DNAS @ Z.30 reasonably red 2:42 pm stan for count



15.43 pm (15 his >643am

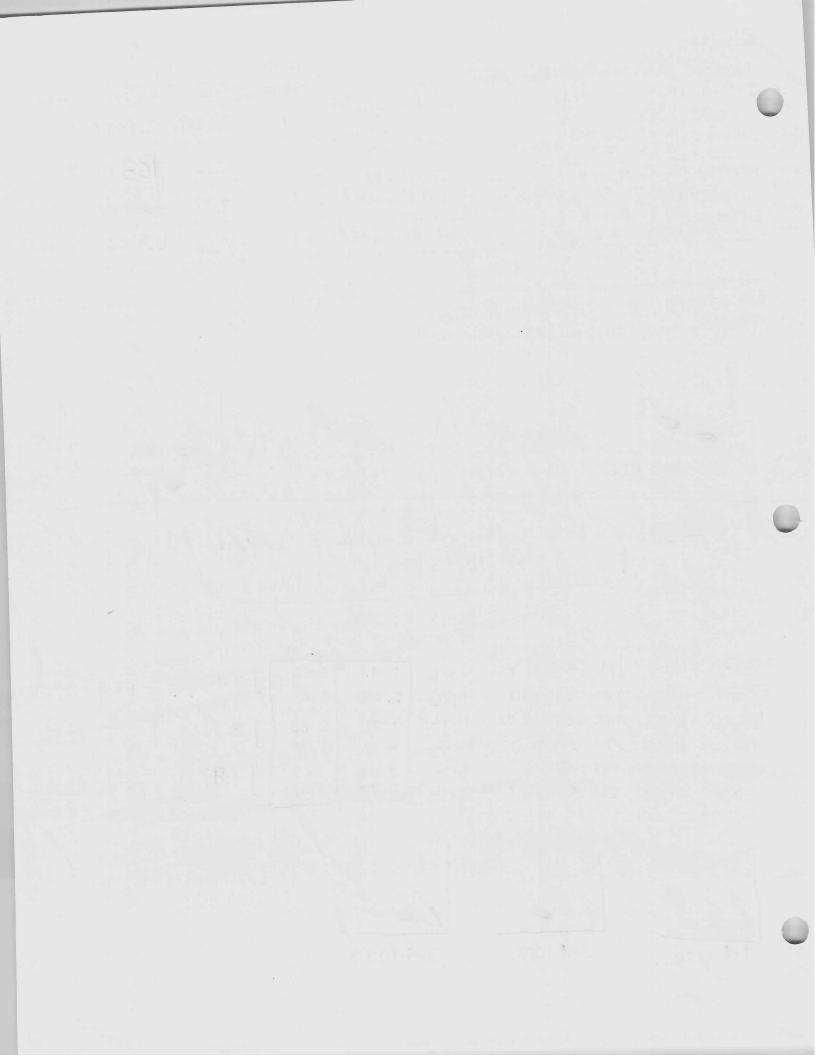
Spin 7:11 (2 wash) 7:38 stanfor conty + M 320 Fo 1068 < 38 yl > 101 2.92 ImL 2.43] 9.10/16.3 = 55.8% recovery 580 C. 1068 218.1pl7 200 5.07 6.18 IML 3/455 = 659/ 480 C. 1073 628N7 IM 1 mL 2.33 1.71 870 C. <11.36 7 1013 200 ImL 4.29 5.78 3/4.05 = 740pl 237.127 300C. 1122 im 1 mL 2.03 3.03 Zan <20.07 3.77 1122 IML 5.46 MSOC. 3/4,245 = 706xl







Incobation @ 08:31 am



		AND UNSTAINED CONTROLS !!!			29 R8	28 R7	27 R6	26 R4	25 R2		23 YG9	1	21 YG5	20 YG3		18 B3	17 B2	16 V15	1		13 V11	12 V10	1	11 V7		10 V/S	V3	9 V3	8 V1	7 UV16	6 U14	5 UV11		3 UV9	2 UV7	# Filter Single color (u) Ref ctrl type name 1 UV2	# F Unmixing ctri
				APC/FIFE 810	APC/Fire 750	20mble NIR	APC-K/00	ARC 570	APC	PE-VIO//0	reicr-cys.5	PerCP Cut F	Pt-Dazzle594	77	Spark blue 550	CICKO FIUUF 488	Alexa Fluor 600	06/30	BV750	BV711	BUCES	BUSOF	RV570	BV510	BV480	Pacific Blue	Pacific Blue	BV4Z1	SORANG	75/Ana	BUV-507	CTOAOG	BUVS63	BUV496	AF	Fluc	in etal
				CD38	CD27	L/D	CD107a	IL2	CD39	HLA-DR	CD26	CD25	TNFα	PD-1	CD3	FoxP3	CCR6	I+Nγ	Va/.2	CCR7	CXCR5		WACHOO	CDAEDA DIOL	CD161 -Biotin (BEAGE)	CD19	CD14	CD127	CD4	CXCR3	V82	CCR4	CD69	CD8	AF-UV6	Marker CD62L	
riew volume /sample	Pippette draw will	R10 Media	Antibody Total	(HIT2)	(0323)		(H4A3)	(MQ1-	(A1)	(L243)	(M-A261)	(M-A251)	(MAB11)	(EH12.2H7	(SK7)	(236A/E7)	(11A9)	(B27)	(3C10)	(G043H7)	(J252D4)		(HI100)	(MEAGOL)	(DEACOL)	(HIB19)	(MSE2)	(A019D5)	(SK3)	(1C6/CXCR3)	(B6)	(161)	(FN50)	(RPA-T8)		Clone Vial Lot #	
e 19.5		305	0.0	-	-	1	6	-	-	-	-	-	+	-	+	-			-			-	_			-		-	-	-	-	-	-	-	-	ot# stim!!!	Dir
							######				1		1	1					1						+	+	1	-	+	1	1	+	-	-	+	3	
/sample	Brilliant Stain	Cumpony rotal	Antihada Tara		<0.000 CT: T >																						1					1				15 min	_
me 58.5	50						+			-					-			-	1000		-	+		1.5						+				1		RT for 10 min	
5	0 150	11.5 34.5		L	Supple Supple			1.2	L		1.2					T.O			4	<2.5> #				2000			1.5	-			<2.5>					30min @37C	H-101-11
G				6				3.6			3.6					4.5	1		u	#####							4.5	4.5			#####					ω	
59.2	50	12.2							8.0		NO.		1.2	1.2				1.2				0./	2		2.0	2.0			<1.3>	0.7		0.5	0.7		1.2	ColdStain 30min @4C	Sport Contract
	150	36.6							2.4				3.6	3.6				3.6	0			2.1		10	6	6			#####	2.1		1.5	2.1		3.6	ω	
																								Strep:												4*C for R	
									L							<u>_</u> .		_			ļ			0.8					_							RBC Lyse, then Fix/Perm	_
20.5	10.6	9.9					<1.2>			2.5	0.5	<1.2>	0.5	0.1	5		<1.5>		0.7									0.5		0.1		4				Intracellular Stain 40 min @RT	
	31.8	29.7					#VALUE!			7.5	1.5	#####	1.5	0.3	15		#####		2.1									1.5				###				ω	

		FBS 59.1	Streptavidin 2.4			1.28 2 Zombie	0.8 3.2 5 PBS	Zombie NIR 4
FBS 28.5 PBS 28.5 PFA 0.06 PBS 0.54		Iotal	Section of the sectio	Number Intracellula SC (29)	Number Surface SCs (26)	Number Instained	Number Samples	
			2	19	w	u	Total	
Perm Water RBC Water	51.3	57	2	19	12	24	FBS	
12.8 115 0.18 1.62		1.8	0	0	0.9	0.9	RBC Lyse	
		27	2	19			Fix	
Fix	115.2	128	16	76	12	24	Perm	
6.75 20.25				0.38		0.09	PFA	

(Act Sch @ 8:12) Spin @ 8:42pm (Cold Sch @ 8:12pm, Spin @ 8:52pm Hot somples @ 8:35pm (no hecolsbut)->9:05an Cold Samples 9:17 pm -> 9:47pm 1005-ce -> Sheet

Simplified Protocol

Thaw cells, DNAse, count.

efectuacend at 1E0mi of R10 rest cells for x12-hrs in a 6-well plate. > Collect, count, aliquot cells 2.E=6 Cells firm polystyrene tibe. Add antigens, stim antibodies and buringStim antibodies in 800 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

800 ul of LiveDead mix (1:2500) @RT for 15min Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min

Wash with 2 ml PBS, spin down 1300rpm 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

Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

Add Streptavidin Mix, incubate @ 4C for 15min

1 ml Nuclear FixPerm, incubate @ 4C for 30min

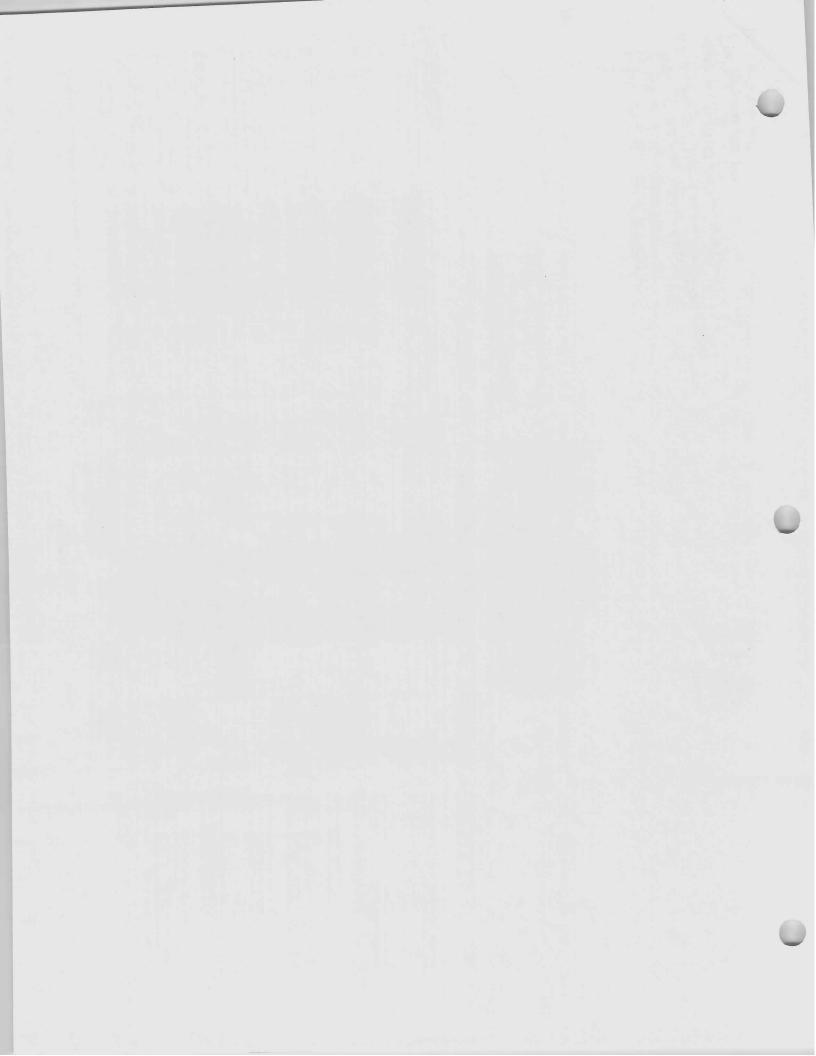
2 ml NuclearWash 1500 rpm 6 min 2 ml NuclearWash 1500 rpm 6 min (vortex every 10 minutes)

First PermWash: Second Perm Wash:

Add Intracellular Stain, incubate @ RT for 40min First PermWash: 2 ml Nuclear Second Perm Wash: 2 ml Nuclear V 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



The across 5 is nowing really load sounds & the plate reader is ferneral, 90 600 373 for unfixed FSC Sethings 600 us 300 373
SSC SSCB

