142-7 a-4	Status Cord Unknown STGH		Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
a-4											
162	- 1011	B, 3	46.8117			2ml	7.9	10.7			
66	Adult			08.12-21		1			*		
-	7001		3347			Iml	11.5	15.8			
			-0-317	1							<u>-</u>
- 4				1 1			_				
										13/11	
	- 1				2						
									-44		
d than	11:57 ac	n								7 0	MA (al
De De Spin C	8:22 8:31	PM -> PM -> 8:380	46 0	'ay				NY 0.24	%.617	0.1	NY062
- hote	9 9:1	8 pm -	7-3500	~ 9.1	topn	14	earl tyle	1,465			
			11/11/1	1 87	Spine 9:	53 V	Ty"	-20pl	20/1		
cs cow	C 9.3) -> .	(9: 55;	sh T		Spive	-	Amine (203)	Wash		
mples	up Uni	72 = 0	Net					TA	31171	Fa/	
56	+ 0	C 9	.49 -7	10:19	pm [Spin @ 10:	190m)			r	
				->2	630 -	> 36,000	4 -7			4/2	î (
1		10.58	Pay			The same of the sa			1=	4 3	2
Con @	36	→	2BC19	see 1	(:Olpm				-1	7 6	
() I	oran c	10:44 p	2						l	7	
dirst	_	10,176	7								
	Inc of the property of the pro	Inc out To Prep & G:40 Inc out To prep & G:40 In down & g Spin & 8:22 D & 8:31 Is finder & Thoto 9:13 Apples we was Maples we was Scis Fax Per 10:26pm —	Inc out 7:49 pm prep @ 6:40 pm n down @ 8:06 pm Spin @ 8:22 pm D @ 8:31 pm is finder @ 8:38p. Is samples @ 9:05 pm shot @ 9:35 mples w vatze q Scis Fax Perm @ 10:53pm 10:26pm > 10:53pm	Inc out 7:49 pm Inc out 7:49 pm pep @ 6:40 pm n down @ 8:06 pm Spin @ 8:22 pm D @ 8:31 pm > 46 pm is findse @ 8:38 pm + samples @ 9:05 pm - 3pm hote 9:18 pm - 9:00 so car @ 9:35 - 10:00 mples up vatz @ 9:49 - 7 Scis Fax Perm @ 10:16 pm 10:26 pm -> 10:53 pm	149 pm Tip Bottem, voiter pulse 8x Inc out 7:49 pm pep @ 6:40 pm n down @ 8:06 pm Spin @ 8:22 pm D @ 8:31 pm > 46 pm is findge @ 8:38 pm + samples @ 9:05 pm - 3pn@ 9:43 pm shot @ 9:35 > 10:00 pm cs con @ 9:35 > 10:00 pm Scis Fax Perm @ 10:16 pm > 2 10:26 pm > 10:53 pm	The out 7:49 pm The Bottem, voiter pilse 8x Inc out 7:49 pm The proper & G:40 pm The down & 8:06 pm Spin & 8:22 pm DO 8:31 pm > 46 pm Is Gidge & 8:38 pm I samples a 9:05 pm - 9:40 pm That & 9:18 pm - 9:43 pm spine 9: That & 9:30 -> 10:00 pm The samples would a 9:40 -> 10:19 pm Scis Factoria & 10:16 pm -> 20:00 pm Scis Factoria & 10:16 pm -> 20:00 pm	Inc out 7:49 pm 300 Inc out 7:49 pm 300 prep @ 6:40 pm In down @ 8:06 pm Spin @ 8:22 pm D @ 8:31 pm > 46 pm Is side @ 8:38 pm I samples @ 9:05 pm 35 pm 9:40 pm I hot @ 7:18 pm - 9:43 pm 5 pine 9:53 Is con @ 9:35 - 9:55 ish 500 Sels Fex Pecar @ 10:16 pm - 26 pm - 36 pm 10:26 pm - 36 pm - 36 pm 10:26 pm - 36 pm 10:58 pm - 36 pm 10:58 pm - 36 pm	Inc out 7:49 pm Inc out 7:49 pm Spin @ 8:22 pm D @ 8:31 pm > 46 pm Is Gode @ 8:38 pm I samples @ 9:65 pm 3pm 9:40 pm I hot @ 7:18 pm > 9:43 pm 5:5100 9:53 I will ge	Inc out 7:49 pm Inc out 7:49 pm The out 7:40 pm The ou	149 pm Tip Bottom, volter pilse 8x Inc out 7:49 pm The	149 pm Tip Bettern, volker pilse &v Inc out 7:49 pm The 0.259.413 The 0.2649.617 The 0.259.413 The 0.259.413 The 0.259.413 The 0.259.413 The 0.2649.617 The 0.259.413 The 0.2649.617 The 0.259.413 The 0.259

Sch stashed @ 11:13 pm Sample Fix Perm 11:17-27-737 Sample 2nd Perm 16:50 pm

12:03 10 min spille	
Introc @ 12:15 am -> 55 am	
505 intra @ 12: el an -> 1:01 am	
SC'S M 57/1 (00/ps) of 0. 40% PFACE	2:32 am
Samples Rented SCS in 45pl of &	D.4% PFX
Done @ 1:12 Rm	Tessena Mellan Kunar Anut
	Ressell babadle
10:34 am sc's (50pl)	0107A -> cord
CD161-cood J gotswitched in acqu	. stopey
CD161	ILT marines not spilled
NY062 mostly 12A-	CD 3 @ 0, 3
11:08 next speed 11:26 all spectral s	es done
Conventional ses @ 11:31 am pra digital pra	-) amino hobe
Damne hydroscop?	
Done @ 12:06 pm/	

Conventional unmixens: not enost UDZ 1/2 CD45RO bright tout - deb as ? 1.98 completely indee

Conv: 8% 4.5% CO3 -> 13.3% 4.6%.
48% 38% gd -> 50% 36.3% Conitral unmix

19/ V24/ GABC 47.2

57 1

60 48 57 39

	2023	
COLD	200	
SOIKE	Chico	

Pippette draw volume /sample 66 59,5	Pippette draw volume /sample 66 59.5	Pippette draw volume /sample 66 59.5	Pippette draw volume /sample 66	Pippette draw volume /sample 66	Pippette draw volume /sample 66	600 ET 150		600 ET 150		19.5		Pippette draw volume /sample	9			
14.5 87 Brilliant Stain 50 300 50.0 300 50	87 Brilliant Stain 50 300 50.0	87 Brilliant Stain 50 300 50.0	87 Brilliant Stain 50 300	87 Brilliant Stain 50	87 Brilliant Stain 50	87	87	155	4.5	-		R10 Media	R		SOLS III	And UNSTAINED CONTROLS !!!
	36 Antibody Total 19.0 114 12.5	36 Antibody Total 19.0 114 12.5	36 Antibody Total 19.0 114	36 Antibody Total 19.0	36 Antibody Total 19.0	36	36		6.0			Antibody Total	Þ			
1.6 9.6										1		НП2	CD38	APC/Fire 810		
7	7	7	7	7	7							0323	CD27	APC/Fire 750		1
-												N/A	L/D	Zombie NIR		
	36	36	36	36	36	36			6.0			H4A3	CD107a	APC-R700		1
7.7	(12)	(12)		<5/100>	<5/100>	<5/100>						3C10	Va7.2/hMR1	AlexaFluor647		
07 43			07									3G8	CD16	APC		1
900			10									PD1.3.1.3	PD1	PE-vio770		
0.5					4.4							MAb11	TNFa	PerCP-Cy5.5		
72					1 2							M-A251	CD25	PE-CY5		
7.2					1.2							M-A261	6770	DE C. S		21 B8
	9	9	9		1.5							TIGI	Scuo.	PE-CFS94		20 B6
7.2			1.2									JAN	NKGOD	PE		19 B4
<5/100> <1.5> <0.25>						<5/100>						1700	cus cus	Spark blue 550		18 B3
1.5 9					1.5							6B11	Va24/hCD1d	FITC/AF488		17 B2
												11A9	CCR6	BV786		
b b												827	IFNY	BV750		
												M-T701	CD7	BV711		15 VII
	+	+	+	+	1.0							G043H7	CCR7	BV650		
												5.1H11	CD56	BV605		
0./ 4.2			0./											BATTAG		
	77	77	77		2							HI100	CD45RA	BV510		1
	2.0	2.0								-		HP-3G10	CD161	BV480		
			2.0							-		SJ25C1	CD19	Pacific Blue		
1.5 9					1.5							MSE2	CD14	Pacific Blue		9 V3
					1.5					-		A019DS	CD127	BV421		8 V1
1.3 7.8			1.3							-		573	CDA	BUV805		7 UV16
0.7 . 4.2 0.25			0.7							-		1C6/CXCR3	CXCR3	BUV737		6 U14
1000	12	12	77		2.0					-		B6	V82	BUV661		TTAN
0.5	0.5	0.5								-		161	CCR4	BUV615		
7.4			200									FN50	CD69	BUV563		
			0.7									RPA-T8	CD8	BUV496		
1,2	2	2	4.4										AF-UV6	AF		2 UV7
77	77		12									SK11	CD62L	004333		
During 6 U015min Tetramer44 HotStain 6 Coldstain RBC Lyse, Spiked 30 min ext 30min @4C F1X/Ferm @RT	6 (RT) Tetramer40 Holstain Coldstain RBC Lyse, min @RT 30min @YC 6 30min @4C 6 then FairPerim	6 (RT) Tetramer 40 HotStain ColdStain min @RT 30min @37C 6 30min @4C	6 L/D 15 min Tetramer 40 HotStain min @ RT 30 min @ 37C 6	6 L/D 15 min Tetramer 40 HotStain min @ RT 30min @37C	6 L/D 15 min Tetramer 40 min @ RT	6 L/D 15 min Tetramer 40 min @ RT	6		During stim!!!		Vial Lot#	Clone	Marker	Fluorochrome	UV2	1 UV2
															Single and Company of the Unmixing ctrl	# []

Simplified Protocol

Thaw cells, DNAss, count.

Collect, count, aliquot cells 2-3.0E+6 Cells R10 / 5ml polystyrene tube Bring volume uplo 1 ml R10, add 2 ul PNACtri and CD107a

Cap and incubate at 37°C for 6 hours

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 HotStain mix, incubate @37C for 30 min

Add Tetramers, incubate @RT for 10 min Wash 2 ml 5% PBS-FBS 1400 rpm, 6 min

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

Add ColdStain mix, incubate @ 4C for 30min Add 300-500 ui 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)

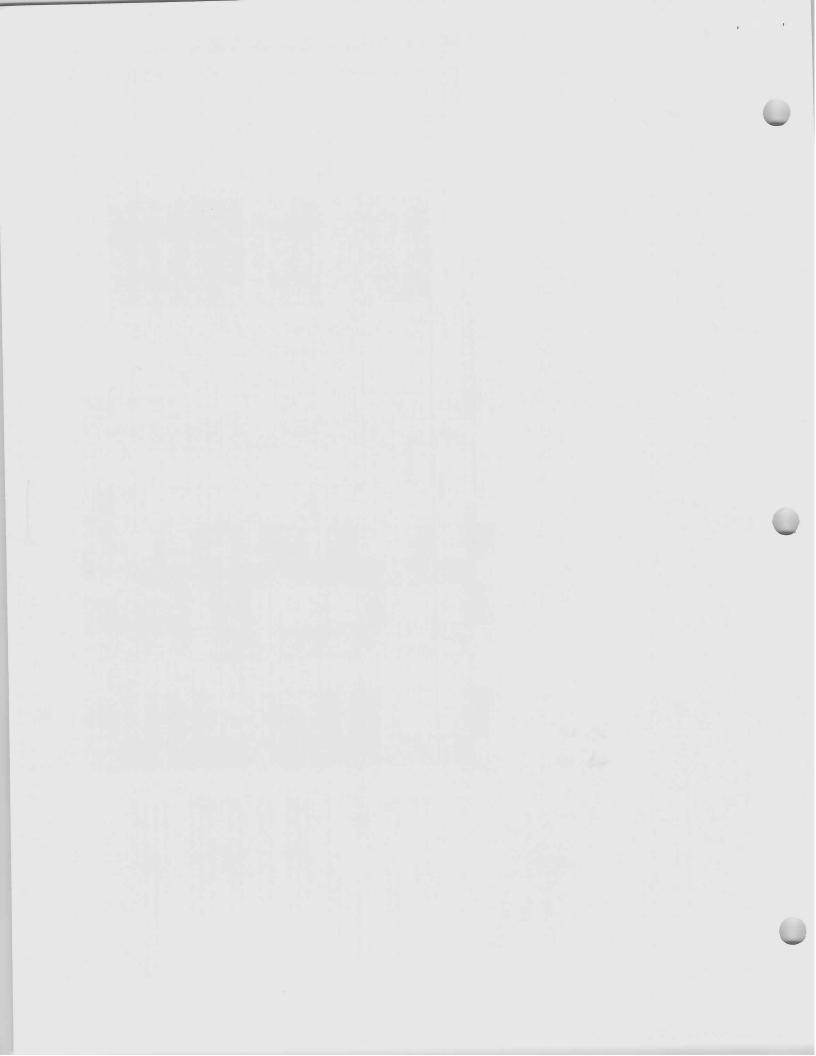
Add Intracellular Stain, incubate @ RT for 40min
First PermWash: 2 ml PermWash 150 First PermWash: Second Perm Wash: 1 ml PermWash 1500 rpm 6 min 1 ml PermWash 1500 rpm 6 min

2 ml PermWash 1500 rpm 6 min

Cap tubes, wrap rack in foil, store at 4*C Resuspend in 70 ul 0.4% PFA.PBS

3 3 (+ NEGAT)

(1 cord colorn)
7 cord chil



1/015min Tetramer 4b Holstain 6		55.75			59.5		66	ume /sample	rippette draw volume /sample		10.0							
Definite Primaria	100	9000		300	50.0	000			-	18	10.5	/sample	ppette draw volume	2				
	1			300	000	200		itain	Brilliant S	87	14.5		10 Media	73		NTROLS !!!	UNSTAINED CON	And
	17.5	1200		75.0	12.5	114		Total	Antibody	36	6.0		ntibody Total	A				
						9.6							НП2	CD38	APC/Fire 810		1	1
						13	7						0323	CD27	APC/Fire 750			2
									<1:2500>				N/A	L/D	Zombie NIR			2
		1402581	9		V1.22			-		36	6.0		H4A3	CD107a	APC-R700			.
				4.2	0.7			<5/100>					3C10	Va7.2/hMR1	AlexaFluor647			۰۱.
Filter Single-color(u) And cell Purmoning and Purmoning	1.0			9.0	1.5								3G8	CD16	APC			١.
	5.0		-	2	1								PD1.3.1.3	PD1	PE-vio770		1	۱.
	1.0	0.5				1.1	7.7						MAb11	TNFa	PerCP-Cy5.5			.1.
		0.0				7.2	17						M-A251	CD25	PE-Cy5			
Filter Singlectolr (u) Red clay Constant Floor celevame Floor ce	1.0					7,0	1.0						M-A261	CD26	PE-CF594			. [.
Filter Single-color (u) Red cyri Durbane Marker Cloine Marker Cloine	1.0			1.1	7.7	0	1						1011	NKG2D	PE			
Filter Single-color(u) Referrit Durachrome Marker Close Color Close Cl		/<0.25>//		7,7	<1.5>			VOOT ICE					SK7	CD3	Spark blue 550			1
Filer Single color(u) Ref cut Marker Clone Marker Marker Clone							1.0	<5/100s					6811	Va24/hCD1d	FITC/AF488			. 1
Filter Single-color(ul) Red cut Unannee and Pourochrome Red Cut Unannee and Pourochrome Red Cut Inches Cobern Red Cut Inches Cut Inc	3.0	1.5				٥	1,7						11A9	CCR6	BV786			. 1
Filter Single-color (uf) Red cut Unanang Ethi Pulporobrome (Marker Clone) REMUSSS COBSL SK11							-						827	IFNy	BV750			1
Filter Single color (u) Ref crit Unmaning crit Indian Ref crit Unmaning crit Indian Ref crit Unmaning crit Indian Ref crit Indian	2.0	P				2	-3						M-T701	CD7	BV711			ı
Filter Single color (u) Ref crit Chimaning Crit Filter Single color (u) Ref crit Filter Single color (u) Filter S						0	1.0						G043H7	CCR7	BV650			1
Filter Single-color (u) Ref crit Unmaring etri Fluorochrome Marker Clone Uval Lots Ethinis 6 (RT) Unmaring etri Fluorochrome Marker Clone (RT) Uval Lots (RT) Unmaring etri Mining etri Mi							400						5.1H11	CD56	BV605		1	J
Filter Single-color(u) Referri Unmaning etri Region (Colore) Referri Value (Clore Region (Clore) Referri Value (Clore) Region				4.2	0.7										afem in			1
Filter Single-color(u) Referri Unmaring Edit Rane Walker Clone Unitary Rane Walker Clone Rane Walker Clone Unitary Rane Walker Clone Rane Walker Clone Walker Rane Rane Walker Clone Rane Rane Rane Rane Rane Rane Rane Ra						12	2					1000	ні100	CD45RA	BV510		1	L
Filter Single color (u) Ref car Durnoxing early Fluorochrome Marker Clone Val Lots Stimular Clone Val Lots Stimular Clone Constain Clone Constain Clone Cl		Ser.		12.0	2.0							1	HP-3G10	CD161	BV480			L
Filter Single color (u) Ref crit Chimania (Line) Colore				12.0	2.0							>	SJ25C1	CD19	Pacific Blue		100	Inc
Filter Single color (u) Ref crit Unmaxing crit Fluorochrome Marker Clone Vial Lots Striit Clone Vial Lots Striit Clone Codstain						9	1.5			I			MSE2	CD14	Pacific Blue			H
Filter Single color (u) Ref crit Unmining early name Lunochyone Marker Clone Vial Lots During string. 6 (RT) L/D15 min min @err Tetramer 40 min @err HodStain min @err 6 coldstain min @err 6 coldstain min @err 6 min @err ABC Lyse, Spiked 30 min @exc Palked 30 min @exc 6 min @err Merr min @err Merr min @err 4 min @err 30 min @exc 7.2	1.0	0.5				9	1.5						A019D5	CD127	BV421			L
Filter Single-color (ul) Red cut/ name Fluorochrome Marker Clone Vial Lots During string 6 (RT) U/D15 min min @RT Tetramer 40 Medisian min @RT 6 Sommin @AC Codstain filter 6 Sommin @AC AC				7.8	1.3								SWS	CD4	BUV805			ı
Filter Single-color (ul) Red cut I agent Fluorochrome Marker Clone Val Lot # During string (RT) Tetramer 40 (RT) Holisian min @37c Codisian filter 6 (RBC Lyss) Splked 30min @47c Plane	0.5	10:25		4.2	0./								1C6/CXCR3	CXCR3	BUV737			1
Filter Single-color (ul) Red cut Unmixing cut Fluorochrome Marker Clone Val Lot # Stimil! 6 (RT) Infire min @RT 20min @30C 6 20min @4C 6 Elde 50 min @4C Elde 50 min @4C 6 Elde 50 min @4C Elde 50 min @4C Eld						12	2.0						86	V82	BUV661		1	1
Filter Single-color (u) Ref ctrl Unmanig ctrl Fluorochrome Marker Glone Vial Lot # During 6 (RT) Internet # Marker 6 (RT) Mining RT 30min @40 6 CoddStain 6 (Rt) Fluorem 6 (RT) Mining RT 30min @40 6 (RT) Mining RT 30min @40 6 (RT) Fluorem 6 (RT) Mining RT 30min @40 6 (RT) Fluorem 6 (RT) Mining RT 30min @40 Mining RT 30min @40 Mining RT 30min @40 Mining RT 30min @40 Mining RT 3	2.0	1		3.0	0.5								161	CCR4	BUV615			1
Filter Single-color(ul) Ref ctrl Unmoving ctrl Fluorochrome Marker Gone Vial Lot # During (RT) International RT John CoddStain (RT) International RT John CoddS				4.2	0.7								FN50	CD69	BUV563			ı
Filter Single-color(ul) Refard Unmoving Earl Fluorochrome Marker Clone Vial Cot # Public 1 (RT) Filter Minigration (RT) Fluorochrome Marker Clone Vial Cot # Public 1 (RT) Fluorochrome Marker Clone Vial Cot # Pu													RPA-T8	CD8	BUV496			1
Filter Single color (u) Ref ctrl Ormx/ng Ctrl Publicheme Marker Clone Vial Lot # stim!!! 6 (RT) min @ RT 30min @37C 6 30min @4C 6 Fix/Perm @RT 10/12 10 10 10 10 10 10 10 10 10 10 10 10 10				7.2	1.2									AF-UV6	AF			1
Filter Single color (u) Ref ctrl Urmx/ng Ctrl Public Processing Colors Walker Clone Walker Clone Walker Single color (u) Ref ctrl Processing Colors Processi		(rixiPerm								20000000		SK11	CD62L	BUV395			1
	2	Spiked 30 min			ColdStain 30min @4C		HotStain 30min @37C	Tetramer 40 min @ RT		No. of Concession, Name of Street, or other party of the Concession, Name of Street, or other pa	The second	Vial Lot#	Clone	Marker	Fluorochrome			1

Simplified Protocol

Thaw cells, DNAse, count.

Collect, count, aliquot cells 2.3.0E+6 Cells R10 / 5ml polystyrene tube Bring volume uplo 1 ml R10, add 2 ul PNACtri and CD107a

Cap and incubate at 37°C for 6 hours

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

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)

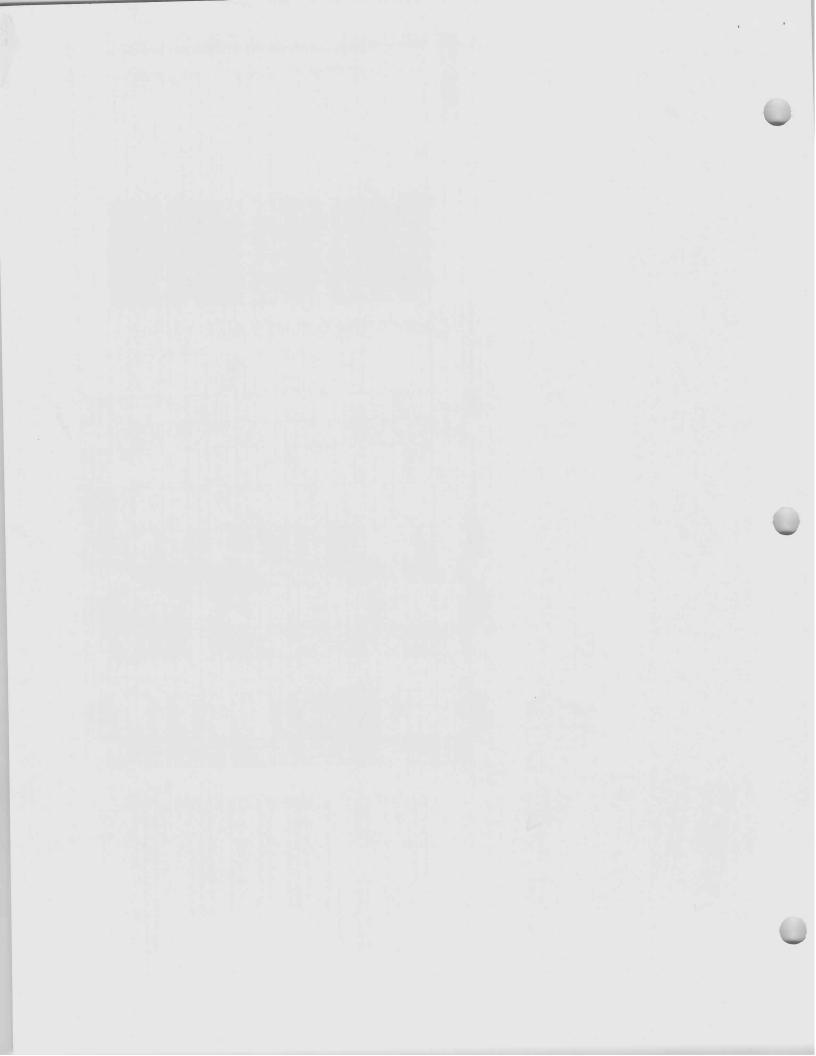
First PermWash: Second Perm Wash: 1 ml PermWash 1500 rpm 6 min 1 ml PermWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min First PermWash: 2 ml PermWash 150 2 ml PermWash 1500 rpm 6 min

Cap tubes, wrap rack in foil, store at 4*C Resuspend in 70 ul 0.4% PFA-PBS

4.51 4.51 20.51 Payumin

all in one Separate



Ex-viv	o gd-CK: P	MA-ionomycin							
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	2	Intracellular 40min @RT	2
1	V450	BV421	PD1			2	4		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD27			1.5	3		
4	B530	Alexa 488	Vδ2			1.2	2.4		
5	B710	PerCPeF710	CD3			1.2			
6	Y590	PE	CD56			0.5	2		
	Y615	PE-Dazzle				0.5	1 6		
7	Y780	PE-Vio770	IFNγ						
8	R670	Alexa 647	TNFα					0.6	1.2
9	R780	APC-Fire750	CD45RO			2		2	4
		7.11 0 7 11 12 7 30		A 1		2	4		
				Antibod	y fotal	8.2	16.4	2.6	5.2
					PBS	12.3	24.6	17.9	35.8
		Pip	oette draw v	olume /	sample	19.5		19.5	



