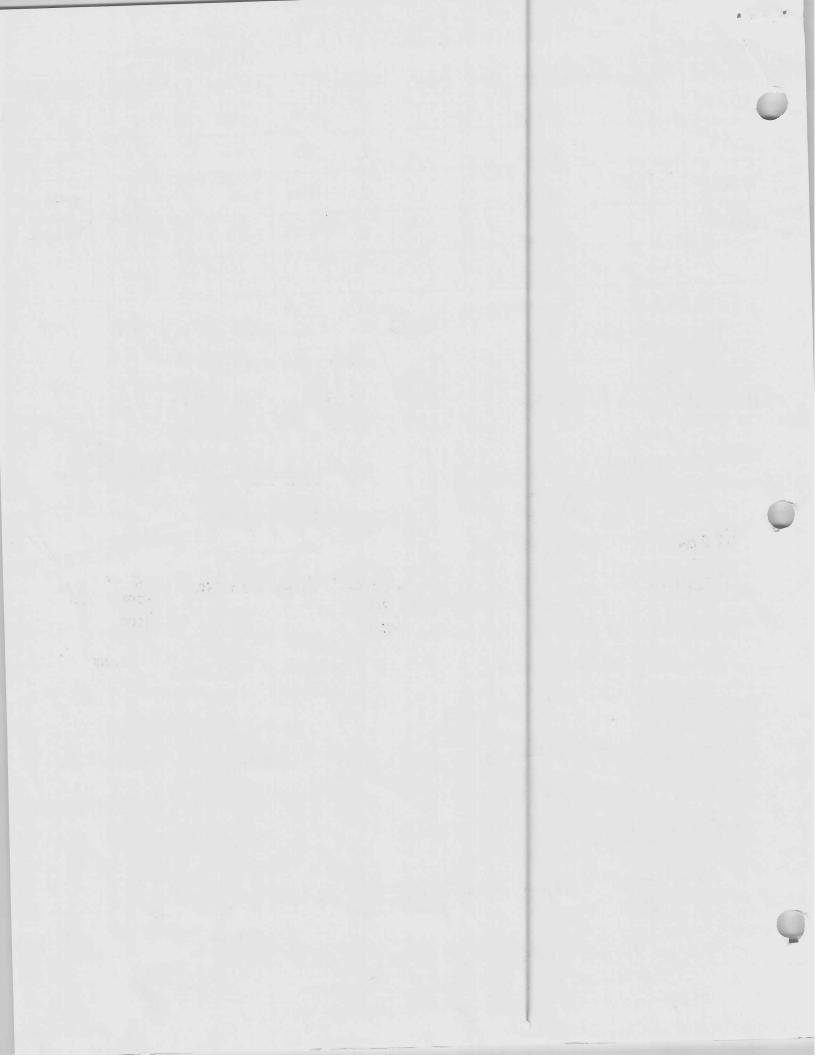
Cold @ 8:38 pm -> [9:08 pa // RBC /yxc



PMA: Kitchen Sink Hot sof a Hot samples spin @ 1400 6min
(RBCal 2ml @8:58pm -> [Cold ses at e 908 pm/ RBc bace 21:10 pm Ses in fridse eqiz spu spin e zilly Tetramers @ 9:42 pm -> 10:22 pm Se tets @ 21:48 pm -> 22:03 pm (spin @ 10:06 pm 5c's Fix Perm @ 21:53 -> 03 -> 13pg 1st yearsh @ 10:18pm Se tet's FixPerin @ 10:18 -> 28 -> 38 pm Jamples + sets
(Zetwash (2nd Permutal) @ 10:32 pm/ 59/53.5 4°C @ 10:50 pm - 11:20 pm RBC 450 @ 11:21pm BUM 50'S @ 11:00 W/40pl D.48/10-18-185 Scs intra @ 11:07 -> (11:07 pm) gpm my 2ml Pernwash Samples RBC/4° spin @[14:26 pm/ 11:38 pm -> 48 -> 58 pm Sis done @ 12:07 am Samples 2nd permissage 12:14 am 545/48 Intracellar @ 12:27 -> 1:07 pm

Final spin @ 1:09 Am Due @ 1:21 pm

Acquisition 8:23pm on 02/09/2023 (Avora 5L install hiccorps disc/our repentedly) event rate 1000 cells for olds. [H2,000 events/sopt CD69 1500 events SSC-A glich is bad 1 col4 TEMRA (CO45RA+ COR7 + CO27-) CO8+ T cells ¿ Set up Peaco ac serapt la se evalution? # NNGZD Food Zambic achally Bol Adult Zambie, A Unstruned sis Hot NY062 2 monogles /Scis done @ 10:13 pm/ (macceptable) Graph and conventional, Useful analysis talk Samples @ 10:21 pm / \* about & explan. Coefficients as comparisons A NDOOG appears fasile (monogle closed vanshed) \$

February 8th, 2023

Coldstain   Rec Lyse, Indian (Coldstain (C		56.5			-											Notes:
Marie   Mari	and the second				60.7		66	ume /sample	ippette draw vol	-	19.5		/sample			
Column   C	8			400		400		Stain	Brilliant	116	14.5	aw volume	Pippette d			
The bolt   Ref	76			109.6	l librarie	152		Total	Antibody	48	0.0	Total	Antibody R10 Media		ONTROLS III	And UNSTAINED C
The control of Ref city   Principle of Ref city   Pr						12.8				3	00					
The interest of the field of						16								APC/I		-
The image of the field   Principle   Pri					1000				-T-2002					APC/I		
1   1   1   1   1   1   1   1   1   1									1.7500				9	Zomi		
	4.0			9.6	1.2					48	6.0			APU		
				5.6	0.7								7.2	Alexa		
	4.0			12.0	1.5								16			
	20.0				1								1			29 R1
	4.0					0.0	7.1					12		DF.		
						9.6	1 2					E		Part		
	4.0		1000			9.6	1.2					(Te	1000	P		1
	4.0			3.0	-	12	1.5					4		PE		
				9.6									1			
1								<: >()						Spark		
1	12.0					12	1.5							Alexa		
	130			STATE OF THE STATE								3				
						8	1					2				
Princip   Prin	8.0					~	-						D7			
Principal Part   Prin		980				0	1.0						CR7			
Priest   P						•	1						D56			
Price   Primary   Primar				5.6	0./											
During   Rept					0,1	-						JUJ	-	900000000000000000000000000000000000000		
				10.0		16	2					J. T.				
During   Retern   Red				16.0	2.0					-		31)	4			1
During   Repter   Primary   Rudrochrome   Marker   Cone   Vial Lot #   Stimit   Repter   Stimit   Repter   Re				16.0	2.0						-	(2)		Pa		
During   Rept   Name   Hudochrome   Marker   Clone   Vial Lot #   Stimin   Rept   Marker   Clone   Marker   Clone   Cl	4.0	ci				12	1.5				-	10.		Pa		1
During   Refer   Name   Rudochrome   Marker   Cone   Vial Lot #   During   Reference   As   Lot #   Lot #   Reference   As   Lot #   Lot #   Lot #   Lot #   Lot #   Lot		O n		and the second second		12	1.5			-	-	DSI C				1
				10.4	1.3						1	21			16	
During   Rectri   Primaria (Marker   Cone   Marker   Cone   Vial Lot # Stimili   Stimili   Rectamer 40   HoStain   Rectuse   Stimili   Rectamer 40   HoStain   Rectuse   Stimili   Rectamer 40   HoStain   Rectuse   Stimili   Rectuse   Rectu	4.0	0.5		5.6	0.7							/9.			1	
During   Prince   Single color (iii)   Ref ctrl   Prince   Prince   Rect   Prince   Rect   Prince   Rect   Prince   Rect   Rec						16	2.0					6)			•	
Name   Color (u)   Ref ctrl   Color   Color   Ref ctrl   Color   Col	8.0	1		4.0	0.5							31)			11	
During   Single coor (u)   Ref ctrl   Comme   Fluorophrome   Marker   Clone   Vial lof #   During   Stimili   Ref   Clone   Vial lof #   During   Ref   Clone   Ref   Clone   Ref   Clone   Ref   Clone   Ref   Clone   Ref   Ref   Clone   Ref   Ref   Clone   Ref   Ref   Ref   Ref   Clone   Ref				5.6	0.7							150)			10	
UV2 Ref ctrl Pluorochrome Marker Clone Via Lot # Stimill 8 (RT) min @RT 30min @37C 8 CodStain min @4C 9.5 Fix/Perm Private 10.00 PR 1.2 9.6 Fi					1							1-18)			9	
UV2 Ref Cttl Charmer Living Fluorochrome Marker Clone Vial Lot # Stim!!! 8 (/RT) min @ RT 30min @370 8 ColdStain then @RT 30min @4C 8 Tai/Perm Price P				9.6	1.2								AF-UV6		n	
UV2    Content					2	550		200000000000000000000000000000000000000				G-56)				
rise single coor (ii) Refett	<b>∞</b>	Spiked 30 min @RT			30min @4C				(RT)	-		$\vdash$			n n	
Color					Coldistain				L/D 15 min	MACHINE S				name	ser Single color (ui)	

But 72.016

Simplified Protocol

Collect, count, aliquot cells 3.0E+6 Cells R10 / 5ml polyslyrene tube Bring volume upto 1 ml R10, add 2 ul PMA/Ctrl and CD107 a Cap and incubate at 37°C for 6 hours

Thaw cells, DNAse, count.

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

Add Tetramers, incubate @RT for 10 min

300 ul BD FixPerm, incubate @ 4C for 20min Wash 2 ml 5% PBS-FBS 1400 rpm, 6min Add ColdStain mix, incubate @ 4C for 30min Add 300-500 ul 1x RBC Lysis for 3 minutes

(vortex every 10 minutes)

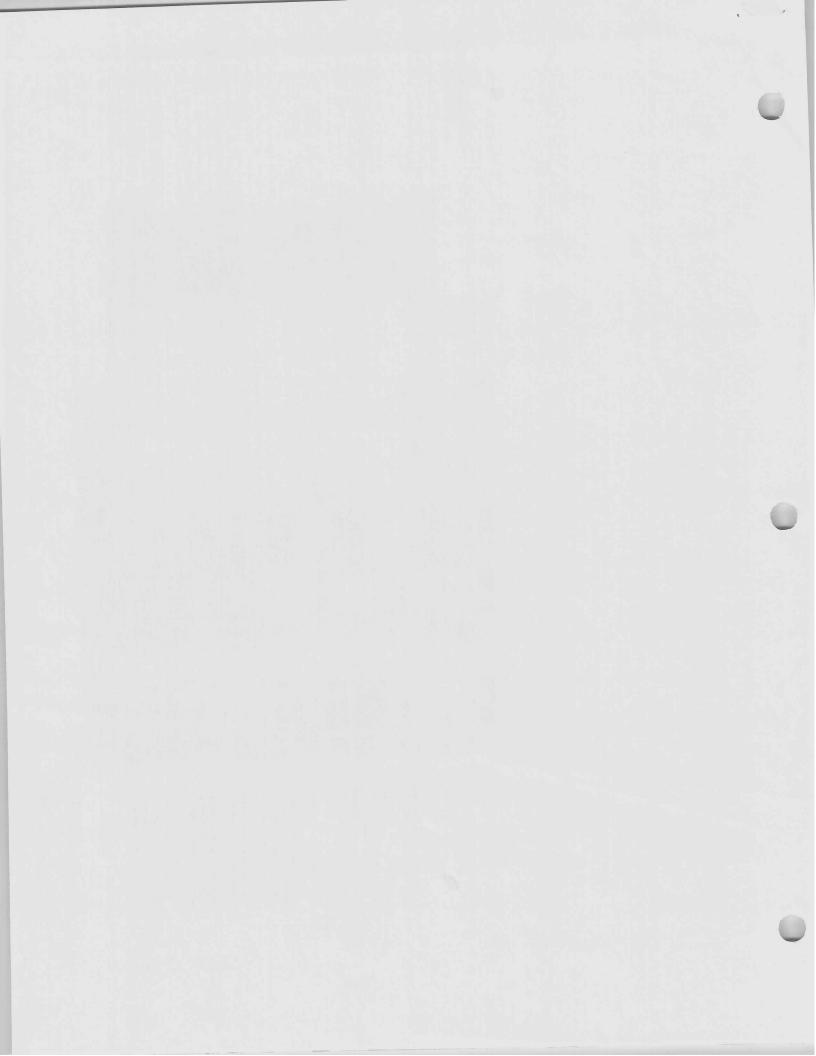
Add Intracellular Stain, incubate @ RT for 40min First PermWash: 2 ml PermWash 15

Second Perm Wash: First PermWash:

1 ml PermWash 1500 rpm 6 min 1 ml PermWash 1500 rpm 6 min

2 ml PermWash 1500 rpm 6 min

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

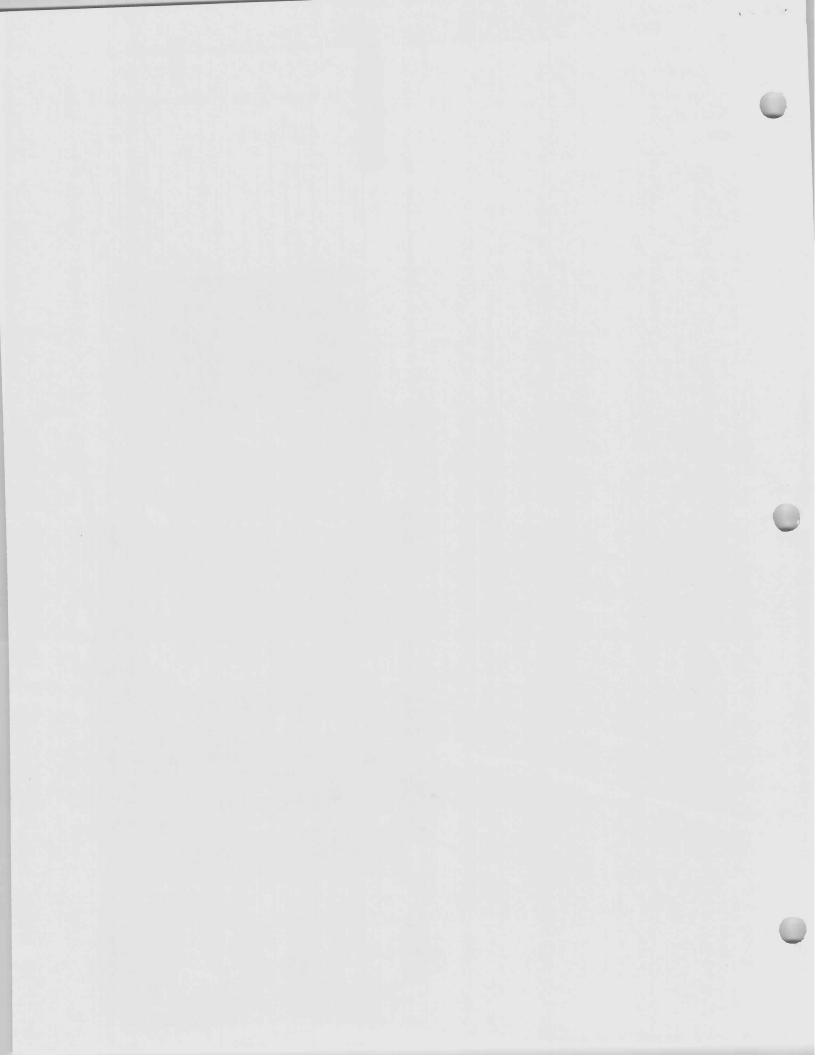


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			And UNSTAINED CONTROLS III		1.800	T <sub>2</sub>			ſ.				1000	E.	Ī				Ė		Single color (ul) Ref ctrl
					1	i i		E PHS	Ē	1		F	5.05		1	110		Callo	1		Ref ctrl
												Land									Unmixing ctrl name
		5			Zombie NIR	AlexaFluor647	APC	PE	Spark blue 550	AlexaFluor 488	BV711	BV650	BV605	BV510	BV480	Pacific Blue	Pacific Blue	BUV805	BUV661	BUV496	Fluorochrome
	/5	2 2	<b>D</b>	40	ا دارا	Va7.2	CD16	NKG2D	CD3	hCD1d	CD7	CCR7	CD56	CD45RA	CD161	CD19	CD14	CD4	V82	CD8	Marker
	/sample	R10 Media	Antibody Total						(SK7)					(Hi100)	(REA631)	(HIB19)	(M5E2)	(SK3)	(B6)	(RPA-T8)	Clone
	Suine																				Vial Lot#
	19.5	20.5	0.0																		During stim!!!
		164	0																		00
	Pippette draw volume /sample	Brilliant Stain	Antibody Total	<1:2500>	^:				<: >												L/D 15 min Tetro (RT) min
	sample				>( )				>()												Tetramer 40 min @ RT
	55	50	8.0		Feb. St.		1.5			L	Ь	1.0		2			1.5				HotStain 30min @37C
		400	64				12			8	00	00		16			12				<b>∞</b>
	56.2	50.0	9.2		WW.	0.7		1.2	<: >(1.2)				0.7		2.0	2.0		0.7	0.7		ColdStain 30min @4C
		400	73.6		36	5.6		9.6					5.6		16.0	16.0		5.6	5.6		<b>∞</b>
																					RBC Lyse, then
	50.5	50	3.5	9	7080		0.5	0.5			1						0.5	0.5			Spiked 30 min @RT
		400	28				4.0	4.0			8.0						4.0	4.0			∞

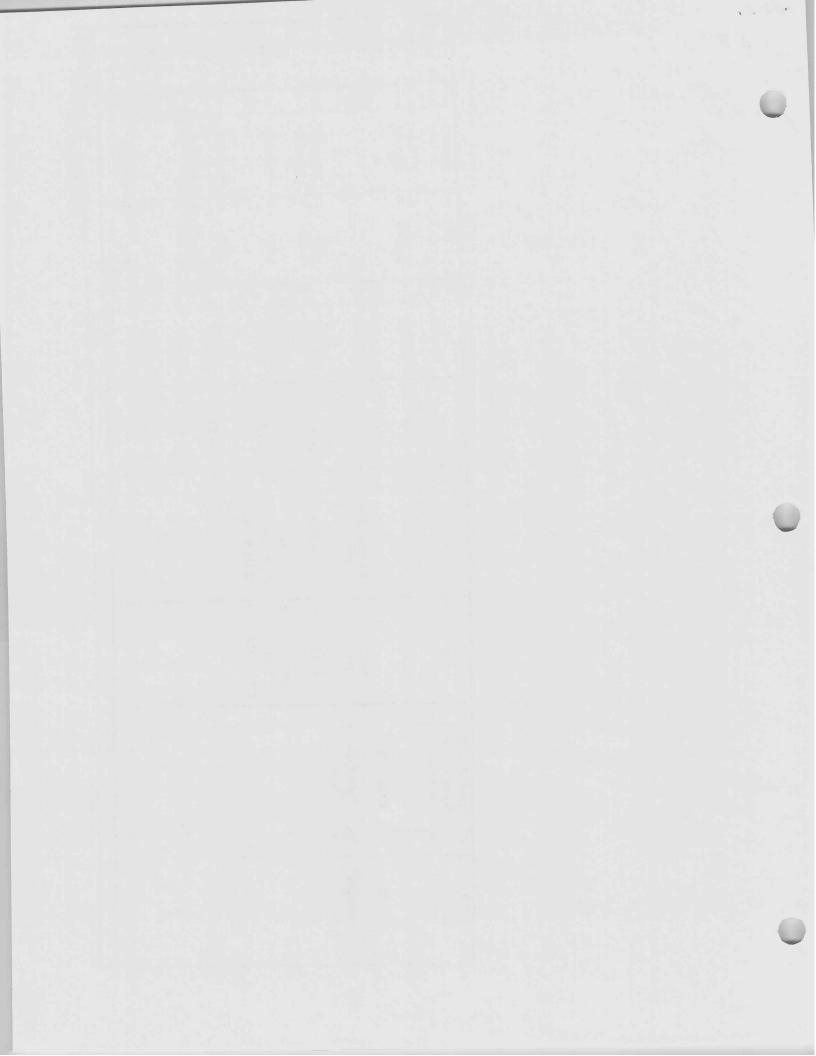
4

10.5

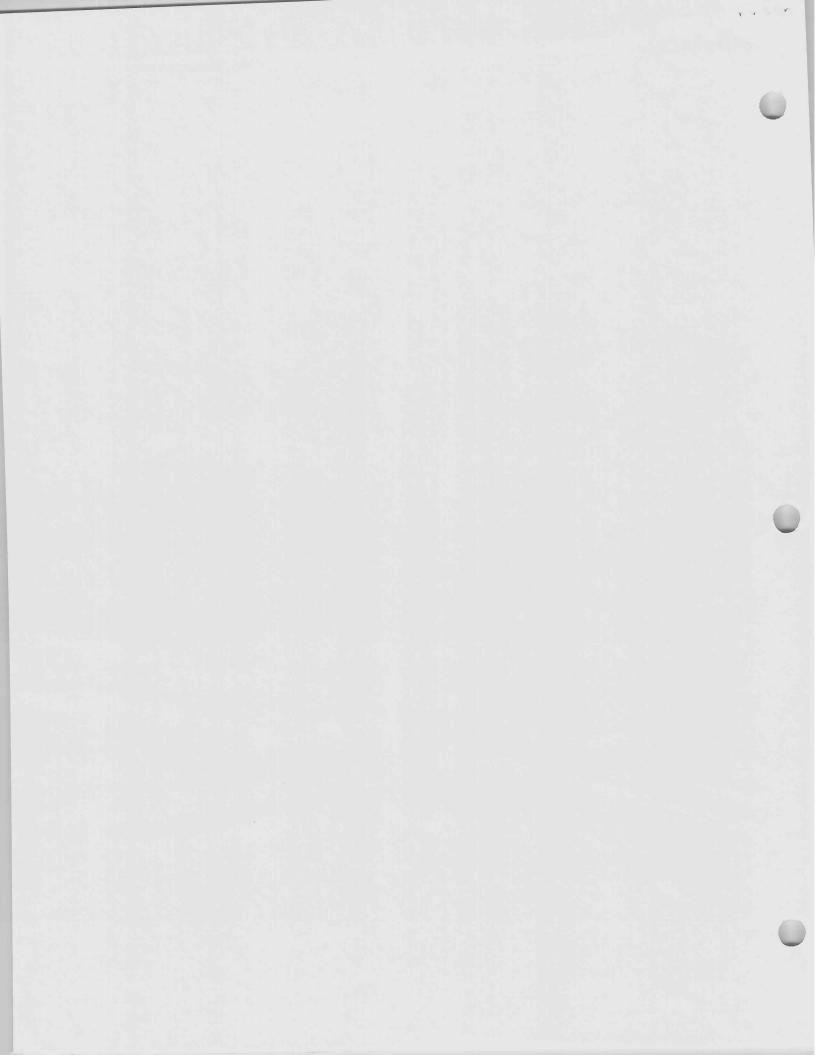


ILT & NK SFC Panel

812	760 783	717 738	697	613 664 679	542 582 598	514 525	473 473	42	388	373
	UV14 UV15	UV13	UV12	UV10	6AN 8AN	UV7	UV5			_
BUV805	BUV737		10	BUV615 BUV661	BUV563	BUV496	AF-UV6		UV2 <b>BUV395</b>	5
[1]	3			[3]	[3]	[2]			[2]	$\vdash$
CD4	CXCR3			CCR4 Vd2	CD69	CD8	AF	, , ,	CD62I	
V15 V16	V14	V13	V12	V10 V11	8 5 6	<b>\</b> 5	≨ <b>≲</b>	<u> </u>		
BV/86	176.15	BV711		BV605 BV650	<b>BV510</b> BV570	BV480	PacBlue	BV421		Violet
<u> </u>	[2.5]	[4]		[3]	[1.5]	<u> </u>	Ξ	4		7
CCR6		CD7		CD56 CCR7	CD45RA	CD161	CD14/19	CD127		
B13 B14	B12	B9 B10	B8	B5 B6 B7	B2 B3 B4	B1				1
Pe-Vio770		PerCP-Cy5.5	PE-Cy5	PE-CF594	AF488/FITC SparkBlue 550 PE				Dide	Rijo
[3]		[2]	[4.5]	4	[1.5] [1]				1	
PD1		TNFa	CD25	CD26	hCD1d/Va24 CD3 NKG2D					
	R6 R5	R4	R2	꼬					1	1
APC-Fire 750 APC-Fire 810	Zombie NIR	APC-R700	AF647	APC					Red	2/1/2023
[2]		[3]	[3.5]	[3.5]						12
CD27 CD38	- <del>3</del>	CD107a	hMR1/Va7.2	CD16						



PMA: Kitchen Sink



PMA: Kitchen Sink

SI: OFF: 51 MBY