Done @ Giolpm V

March 10th	n, 2025				% % <b>*</b> <del>*•</del>				
Specimen	Status	Location	Conc	Date	Notes	Volume	CD 45.	1	HN/mL
Inf 1127-1	HU81 cord val	Bax 29 A			Trothymes ex.	Volume	CD45+	Total	Resuspension
Inf/283-4	4 406	Box BK.						5.1M	10.27
	Adolf	H15 ~	15 E G	319/23	Lysed	0.5		team	
	Adult							14M	72.5
1 10050 1 10050	sels	P	12 € 6	5/13/85		1		7M	10.75
a- 2	and					1		1140	12.5
In + 1334 as	Than	Box lat !	3.	Pres	e of Strofamin R	0.5		nur	71 , 34
5:31 pm 5:45 PM	First s	spms	H mor	9	e of Styrofaam in Ricing for backep	10	& VD602-	2W	PDE SHYM
GIOZ DNAS	Se and	Spin					Comoca	~	~ ~ ~
641 Second	Set				3.5	195pl			t M each
7:49 Con	The same of the sa	Its into	in had	A T	<u>*</u>	a.7.	Infa6		orm ————————————————————————————————————
2:11 Am :	Incobor	ction start	1	[6"]	1 x 306-9	14		J	
3:59 Gdg1	iPlus mo	cst			217		NDOSC		Helach IMSEB sels
H:49 AM									God of 1 ses
11:52 AM	Reage	ints preppe	ed a	37/48	ed by		Inf		05.1 M Scs
700 771	· Spart	Lam			:				3.(1, 300
12:427	MZ	NIR add	ded		A				
1216	Hot So	d water	31:14	1					
1:17 5	iamples:	378 Wash	@ 1:29	(Pm					
1:23 Z	ic Au	Mix don	0 1.4	15pm	14				
1.56	cold sc	5	-		<b>.</b>				
2-29 pm	m RBC	lyse -	en'n @	2:330	n				
1.	- recpi	raid ain			·				
2.59 pm	n Street	t wash, wil	11 dok	08/50	onland septek				
(5:08			/		acum?				
Time 3:	36 pr	n -> (	06-	7 16	-> Zg				
2nd	rach (8	4:31 F	m						
5:00 FC SI	wash	0 4140	priv		*				
5:41 pm 3 8d		rart							
Donna	0				.# · · · · · · · · · · · · · · · · · · ·				

2018-06-13 -> 2018-08-17 40

12:42 PM 3 NERODECS

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March 11th, 2025

29 R1 30 R2 R3 31 R4 R5 32 R6 33 R7 34 R8 and UNSTAINED CONTROLS !!! 1 UV1 1 UV2 1 UV7 2 UV7 2 UV9 3 UV10 4 UV11 5 UV14 6 UV16 6 UV16 7 V2 8 V3 V4 5 Filter Alexa Fluor 647 Zombie NIR APC-Fire 750 APC-Fire 810 Spark Blue 574 PE-Fire 700
PE-Fire 744
PE-Vio 770
PE-Fire810
APC PE-Dazzle 594 BUV563 BUV615 BUV615 BUV737 BUV805 BUV805 BV421/ Sbark Violet 423 Pacific Blue APC-R700 PE-Cy5 Fluorochrome RB780 PE RB705 RB613 BV605 BV650 BV711 BV750 BV786 BV570 BV480 tood und CD107a CD127 CD25 HLA-DR CD56? CD39 IL2 CD27 CCR7 CXCR3 TNFa CXCR5 ICOS CD45RA CD8 Va7.2 IFNV CCR6 CD26 FoxP3 CD3 CD45 PD1 CD161 CD28 CD14 CD19 Marker CD62L CD69 CCR4 V82 CD38 Cods Supernatant Pippette draw volume 9 (REA631 Antibody Total R10 Media Clone Beads Dose 1.2 1.2 19.3 19.5 During stim!!! 6 Antibody Total Brilliant Stain 5 <1:2500> Pippette draw L/D RT 15 min RT for 10 min 11.4 22.8 31.2 Hot Stain 30min @4C 1.2 1.5 57 114 6 6 G S 7.5 6 7.5 16.8 33.6 **47.4** ColdStain 30min @4C 0.6 <1.5> 1.5 1.5 1.2 0.6 1.5 1.5 1.0 0.9 0.5 1.2 84.0 168 7.5 7.5 5.5 4.5 wo 7.5 2.5 Strep <0.4> 4\*C for 15 min RBC Lyse, then Fix/Perm αβ T cell SFC panel Intranuclear Stain 40 min @RT 9.5 19 **25.5** 1.2 109 1.5 1.5 0.8 47.5 95 1.5 248 6 1 15 7.5 0.5

t as whose ad

Wash with 2 ml PBS, spin down 1300rpm 8min 800 ul of LiveDead mix (1:2500) @RT for 15min Wash 1.5 ml 5% PBS-FBS, spin 1300 rpm, 8min

Add CD161 biotin antibody for 10 minutes at RT

Add HotStain mix, incubate @ 37C for 30min Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min

Add ColdStain mix, incubate @ 4C for 30min Add 300-500 ul 1x RBC Lysis for 3 minutes Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min

Add Streptavidin Mix, incubate @ 4C for 15min Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min 0.8 ml Nuclear FixPerm, incubate @ 4C for 30min (vortex every 10 minutes)

First PermWash: 1.5 ml NuclearWash 1500 rpm 6 min

Second Perm Wash: 1.5 ml NuclearWash 1500 rpm 6 min

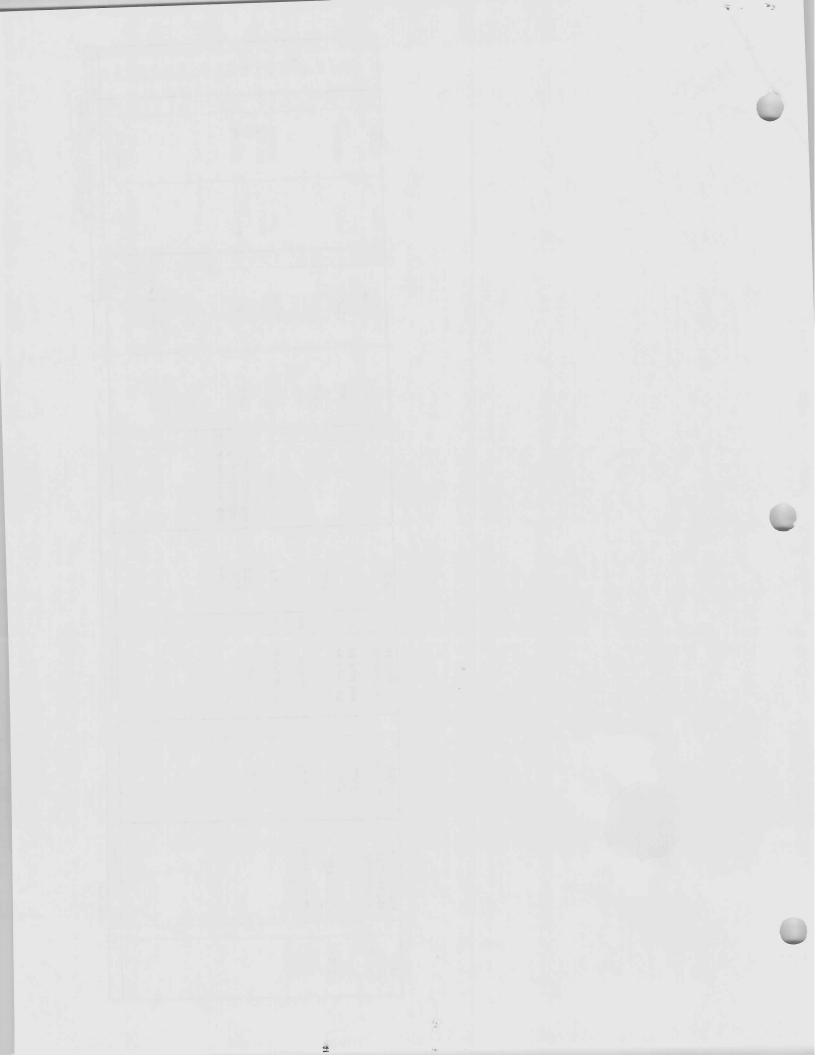
Add Intracellular Stain, incubate @ RT for 40min First PermWash: 1.5 ml NuclearWash 1500 rpm 6 min Second Perm Wash: 1.5 ml NuclearWash 1500 rpm 6 min

Resuspend in 80 ul 0.4% PFA-PBS Cap tubes, wrap rack in foil, store at 4\*C

80 M = (3 ms.) 8 1Zi 100 (6) \* 800 pt = 4.8 ml SC 280pl \* 38 a.5 +5 South \* 5 = 2.5 1m2 x 5 = 50 m2 5 x 300 pt = 1.5 ml 150 + 1.35ml DI water 25.2 ml 1.5ml \* H \* 5 = 30ml 1m1 \* 38 = S00 \* S \* 4 = 10 580 ×33 × 2 = 83 38ml 24 ml 68 mc) 5% FBS-PBS 63 MLS Franklost Grish TZ/L ZOMBIE NIR Pix 3ml 5m1 883

Alpha Beta T Cell SFC Panel

									-	_
			697 717 738		582 598	514 525	458 473 508	428 443	388	373
	UV15 UV16	UV14	UV12 UV13	UV10 UV11	6/10	UV7	UV6	UV3	UV2	<u> </u>
	BUV805	BUV737		BUV615 BUV661	BUV563	BUV496	cFluor UV440		BUV395	Spark IIV 387
	CD4	CD38		CCR4 V82	CD69			Ĭ	CD62L	
V 10	V15	V14	V12 V13	V10 V11	\$ 8 5	<b>6 √</b> 5	\$ \$ \$	5 ≤		t
LIVEDEADNIR786	BV786	BV750	BV711	BV605 BV650	BV570	BV480	Pacific Blue	Spark Violat App		Violet
B14	CCR6 B13	INF <sub>Y</sub>	88 Va7.2 B10	CD45RA E	CD16	CD161	CD28 CD14/CD19	}		
4	ω Ν		B8 B9 B10	B6 B7						8
RB780	RB744		SBB8775 NaChorships 800 RB705	RB613	Spark Blue 574					Blue
CXCR5			CD26	PD1	CD3 CD45					
YG10	χ <sub>α</sub> :	YG7	YG5 YG6	YG2 YG3	YG1				1	1
PE-Fire810	Pa-Vio770	PE-Fire 700	PE-Cy5	PE-Dazzle594	PE				rellow-Green	V-II
CD56		CD127	CXCR3	TNFα	icos					
R7	R	7, 7,	22 22 22			***************************************			1	
APC-Fire 750	Zombie NIR	APC-R700	APC Alexa Fluor 647						Red	3/11/2025
CCB7	Viability	CD107a	CD39 IL-2							



March 10th, 2025

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UV70-UV8-0 tag addhor

CD6a, caR4, coB,

major on Pacthe (
CD16)

CD45

CD8

FORPS Span

