April 3rd, 2023

"Work_GodDamn_IT!"

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
NY062	Adult					1.5	5.63	8.22 E6	8.44		
Inf/142-1 a-3	Study reject 5766					1.5	8.94	1.36 E7	13.41		

12:04 62 thawed
12:06 cord
12:15 DNAse
12:25 DNAse cord
12:34 count stain

530µl + 27
280µl + 402
2.00 (C)
1.32 (M)

Inoculation @ 1:32 pm

Spin @ 7:35 pm
8:05 L/O

Sc's @ 20:13 → 33pm

Samples stashed @ 8:41 pm
9:30pm (conventional Abs prepped, thrown at R tunnel!)
21:45pm

21:51 pm → 37°C → 10:21 pm
& Surface → 10:11 pm
<Abs done @ 10:06>

Strep @ 22:27 pm → 42pm
tets @ 22:36 pm → 11:16pm
→ + RBC lyse for all samples @ 22:39pm
Sc's 0.4% PFAed w/100µl @ 22:48 pm
+R

Fix Perm @ 22:56 — 06 — 16pm
Spin Tets & ~~Ant~~ Fix Perm @ 23:22

NY062 cord
Spectral
P1 P2 Pab CC ctrl CC FMA cord

NY062 for sc's

42 × 4 = 168µl + 10µl = 178µl
40µl / 1.32
5.28µl

40µl × 2
120µl RC × 6µl

800µl × 4 = 3200µl L/O Aqua

1000µl L/O Heaven

1600µl CONVER

Cold @ 11:36 pm → 12:06 am
RBCed @ 00:08 am
Intra @ 11:43 → 12:23am

Spilled cord ... F! 00:15am

Fix Perm @ 00:24 am → 34 → 44

Intrac @ 0:07pm

Final spin @ 1:40pm

Done @ 2:01 am

April 3rd, 2023

Ex-vivo Gd-P4b

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	1	Intracellular 40min @RT	1
1	V450	BV421	PD1			2	2		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD56			1.8	1.8		
4	B530	Alexa 488	Perforin					3	3
5	B710	PerCPeF710	CD3			1.5	1.5		
6	Y590	PE	GzmB					1.5	1.5
	Y615	PE-Dazzle							
7	Y780	PE-Vio770	NKG2A			0.6	0.6		
8	R670	APC	Vδ2			1	1		
9	R780	APC-Fire750	CD16			1	1		
					Antibody Total	7.9	7.9	4.5	4.5
					PBS	12.6	12.6	16	16
					Pippette draw volume / sample	19.5		19.5	

6M

Ex-vivo Gd-P2

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	1	Streptavidin	Intracellular 40min @RT	1
1	V450	BV421	Vδ2			1	1			
2	V525	BV510	CD3			1.5	1.5			
3	V670	BV650	NKG2D			2	2	1.5		
4	B530	Alexa 488	Perforin						3	3
5	B710	PerCPeF710	CD56			1	1			
6	Y590	PE	PD1			1.5	1.5			
	Y615	PE-Dazzle								
7	Y780	PE-Vio770	NKG2A			0.6	0.6			
8	R670	APC	Vδ1			1	1			
9	R780	APC-Fire750	L/D Horizon		<1:1000>					
					Antibody Total	8.6	8.6	1.5	3	3
					PBS	11.9	11.9	19	17.5	17.5
					Pippette draw volume / sample	19.5		19.5	19.5	

Ex-vivo Gd-P1

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	1
1	V450	BV421	PD1			2	2
2	V525	BV510	L/D Aqua		<1:500>		
3	V670	BV650	CD16			1.5	1.5
4	B530	FITC	Vδ2			1.2	1.2
5	B710	PerCPeF710	CD25			2	2
6	Y590	PE	CD28			2	2
7	Y615	PE-Dazzle	CD27			1.5	1.5
8	Y780	PE-Vio770	CD3			0.5	0.5
9	R670	APC	Vδ1			1	1
10	R780	APC-Fire750	CD45RA			1.8	1.8
					Antibody Total	13.5	13.5
					PBS	7	7
					Pippette draw volume / sample	19.5	

Ex-vivo gd-CK: PMA-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	1		
	Y615	PE-Dazzle							
7	Y780	PE-Vio770	IFNγ					0.6	1.2
8	R670	Alexa 647	TNFα					2	4
9	R780	APC-Fire750	CD45RO			2	4		
					Antibody Total	8.2	16.4	2.6	5.2
					PBS	12.3	24.6	17.9	35.8
					Pippette draw volume / sample	19.5		19.5	

April 3rd, 2023 Sealed Tetramers

#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	2	L/D 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @37C	2	ColdStain 30min @4C	2	RBC Lyse, then Fix/Perm	Spiked 30 min @RT	2
1	UV2				BUV395	CD62L	SK11								1.2	2.4			
2	UV7				AF	AF-UV6													
3	UV9				BUV496	CD8	RPA-T8								0.7	1.4			
4	UV10				BUV563	CD69	FN50								0.5	1.0		1	2.0
5	UV11				BUV615	CCR4	1G1						2.0	4					
6	U14				BUV661	Vδ2	B6								0.7	1.4		0.5	1.0
7	UV16				BUV737	CXCR3	1C6/CXCR3								1.3	2.6			
8	V1				BUV805	CD4	SK3						1.5	3				0.5	1.0
9	V3				BV421	CD127	A019D5						1.5	3					
	V3				Pacific Blue	CD14	M5E2								2.0	4.0			
10	V5				Pacific Blue	CD19	SJ25C1								2.0	4.0			
11	V7				BV480	CD161	HP-3G10						2	4					
					BV510	CD45RA	HI100								0.7	1.4			
12	V10				BV605	CD56	5.1H11						1.0	2					
13	V11				BV650	CCR7	G043H7						1	2				1	2.0
14	V13				BV711	CD7	M-T701						1	2					
15	V14				BV750	IFNγ	B27											1.5	3.0
16	V15				BV786	CCR6	11A9						1.5	3					
17	B2				FITC/AF488	Va24/hCD1d	6B11												
18	B3				Spark blue 550	CD3	SK7								1.2	2.4		0.5	1.0
19	B4				PE	NKG2D	1D11						1.5	3				0.5	1.0
20	B6				PE-CF594	CD26	M-A261						1.2	2.4					
21	B8				PE-Cy5	CD25	M-A251						1.2	2.4				0.5	1.0
22	B10				PerCP-Cy5.5	TNFα	MAb11											2.5	5.0
23	B13				PE-vio770	PD1	PD1.3.1.3								1.5	3.0		0.5	1.0
24	R1				APC	CD16	3G8								0.7	1.4			
25	R2				AlexaFluor647	Va7.2/hMR1	3C10												
26	R4				APC-R700	CD107a	H4A3		6.0	12									
27	R6				Zombie NIR	L/D	N/A				<1:2500>								
28	R7				APC/Fire 750	CD27	O323						2	4					
29	R8				APC/Fire 810	CD38	HIT2						1.6	3.2					
And UNSTAINED CONTROLS !!!							Antibody Total		6.0	12	Antibody Total		19.0	38	12.5	25.0		9	18
							R10 Media		14.5	29	Brilliant Stain		50	100	50.0	100		50	100
							Pippette draw volume /sample		19.5		Pippette draw volume /sample		66		59.5			56	

41.5

Simplified Protocol

Thaw cells, DNAse, count.
Collect, count, aliquot cells 2-3.0E+6 Cells R10 / 5ml polystyrene tube
Bring volume upto 1 ml R10, add 2 ul PMA/Ctrl 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
Wash 2 ml 5% PBS-FBS 1400 rpm, 6 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: 1 ml PermWash 1500 rpm 6 min
Second Perm Wash: 1 ml PermWash 1500 rpm 6 min

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

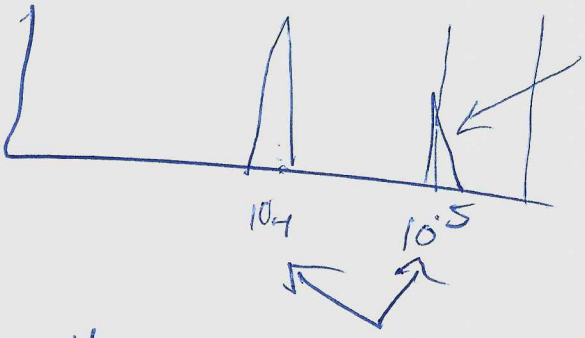
1.5/1.2 antibody

April 3rd, 2023

Aurora @ LSR 2:44 pm

2021 11 24th

		2nd	3rd
FSC	570	700	730
SSC	300	300	300
	555	555	555
	535	580	580 R780
	565	610	410
	525	525	525
	475	475	475
	550	520	520
	540	540	540 P4b
	640	640	640
	650	650	650
	530	580	680



w/ Zombie Horizon

530 → Perforin (10^5)

$10^4 - 10^2$

P4b V525 - V670

R780 - Y780

V525 * V430

(PE Y590 too high)

4.75	0.50
20	16.5
	2.79
	11.9

CM V525 * / V670

R670 - V670

V450 * V525

R610 * R780 ← affected in #2

R780 * Y780

P2 (w/ wrong L/D)

R780 * Y780

Y590 * Y780

(P1 w/ wrong L/D)

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