

April 12th, 2023

1:00

Spiking in Cord

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
Inf 42-7 a-4	Cord Unknown STGH	Box 5 A, B,3	6.8 pl			2 ml	7.9	10.7			
N462	Adult		20 EG ml	08-12-21		1 ml	11.5	15.8			

1450 pl
80
4600 pl
40 pl

Cord thaw 11:57 am
stach @ 12:25

1:49 pm Tip Bottom, vortex pulse 8x

Inc out 7:49 pm

Ab prep @ 6:40 pm

Spin down @ 8:06 pm

Sc's spin @ 8:22 pm

WD @ 8:31 pm → 46 pm

Sc's bridge @ 8:38 pm

Hot samples @ 9:05 pm → 9:40 pm

Sc hot @ 9:18 pm → 9:48 pm

Cold sc's 9:30 → 10:00 pm

sc's cov @ 9:35 → 9:55 ish

Samples w/ V472 @ 9:44 → 10:19 pm

Sc's Fix Perm @ 10:16 pm → 26 pm → 36 pm

Samples old 10:26 pm → 10:58 pm

Cov @ 36 → RBC lysis @ 11:01 pm

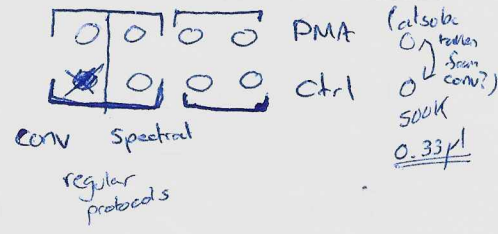
Sc's first perm @ 10:44 pm
2nd @ 59 pm

Sc's stashed @ 11:13 pm

Sample Fix Perm 11:17 → 27 → 37

Sample 2nd Perm 11:50 pm

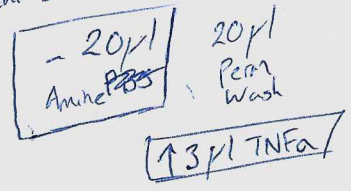
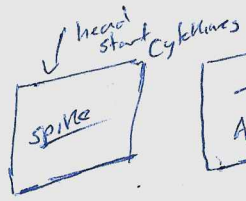
300 2 ml
+ 7 x 2 pl
5600



Inf 0.250% 413 0.06
NY 0.240% 677 0.104

1.32
x 5
6.60 pl stimulat

1.83 pl
x 3 pl
5.49



↑ 3 pl TNFa

4 1/2 11
3 2
7 9

20.5 15 x 6 = 9
- 2.7 12 x 6 = 7.2
17.8 x 6 = 106.8

12:03 10 min spike

Intra @ 12:15 am \rightarrow 55 am

SCS intra @ 12:21 am \rightarrow 1:01 am

SC's in 57 pl (coops) of 0.49% PFA @ 12:32 am

Samples intra SCS in 45 pl of 0.49% PFA

Done @ 1:12 am

Tessana McManus
Kumar Amit
Russell Gabrielle

10:34 am SCS (50 pl)
<150>

CD107A \rightarrow cord

CD161-cord \rightarrow got switched in acqu. setup

CD161

N4062 mostly RA—

ILT markers not spiked

CD 3 @ 0, 3

11:08 next speed

11:26 all spectral SCS done

Conventional SCS @ 11:31 am

Samples @ 11:43 am

pna ^{degam} ~~157~~ \rightarrow amine-like

Δ amine hydroscop?

Done @ 12:06 pm

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Conventional unmixing: not exact VD2 fit
CD45RO bright tail - debris? 1.98 complexity index

Conv: 8% 4.5% CD3 → 13.3% 4.5%
48% 38% gd → 50% 30.3%

@ initial unmix

19/ Vaz4/KABC Vaz.2

50

60 48
51 39

#	Filter	Single color (ul)	Ref ctrl	Umling ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	6	U/D 15 min (RT)	Tetramer/40 min @ RT	HoeStain 30min @37C	6	ColdStain 30min @4C	6	RBC Lysate then FxRperm	Spined 30 min @RT	2
1	UV2				BUV395	CD82L	SK11												
2	UV7				AF	AF-UV6													
3	UV9				BUV496	CD8	RP4-18												
4	UV10				BUV563	CD69	FN50												
5	UV11				BUV615	CD64	1G1												
6	UV14				BUV661	W2	B6												
7	UV16				BUV737	C/CKR3	1C6/C/CKR3												
8	V1				BUV805	CD4	SK3												
9	V3				BU421	CD127	A01955												
10	V3				Pacific Blue	CD14	M5E2												
10	V5				Pacific Blue	CD19	S12C1												
10	V5				BU480	CD161	HP-3G10												
11	V7				BU510	CD45RA	HL100												
12	V10				BU605	CD56	5.1H11												
13	V11				BU650	CCR7	G043H7												
14	V13				BU711	CD7	M-7701												
15	V14				BU750	IRP1	B27												
16	V15				BU786	CCR6	11A9												
17	B2				FTC/AF488	VA24/ICD1d	6B11												
18	B3				Spark blue 550	CD3	SK7												
19	B4				PE	NG2D	1D11												
20	B6				PE CF594	CD26	M-A261												
21	B8				PE-Cy5	CD25	M-A251												
22	B10				PerCP-Cy5.5	THr4	MAH11												
23	B13				PE-vio770	PD1	PD13.13												
24	R1				APC	CD18	3G8												
25	R2				AlexaFluor487	VA1.2/HR1	3C10												
26	R4				APC-R700	CD107a	H4A3												
27	R6				Zombie NIR	U/D	N/A												
28	R7				APC/Fire 750	CD27	O323												
29	R8				APC/Fire 810	CD38	HT2												
And UNSTAINED CONTROLS III										R10 Media									
										Pipette draw volume /sample									
										6.0	36	Antibody Total	19.0	114	12.5	75.0	8.75	17.5	
										14.5	87	Brilliant Stain	50	300	50.0	300	50	100	
										19.5		Pipette draw volume /sample	66		59.5		55.75		

Simplified Protocol

Thaw cells, DMSO, count.
Collect, count, aliquot cells 2.3.0E+8 Cells R10 / 5ml polypropylene tube
Bring volume up to 1 ml R10, add 2 ul PMACRI and CD107a
Cap and incubate at 37°C for 6 hours

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

Add HoeStain 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) cord colt-1
7 cord colt
5 adult AMR
3 3
9 (KFC41)

#	Filter	Single color (U)	Ref ctrl	Urnking ctrl name	Fluorochrome	Marker	Clone	Vial lot #	During stimuli	6	L/D 15 min (RT)	Tetramin 40 min @ RT	HeadsStain 30min @ 37C	6	ColdStain 30min @ 4C	6	RBC Lysis then FixPerm	Spiked 30 min @ RT	2
1	UV2				BUV935	CD62L	SK11								1.2	7.2			
2	UV7				AF	AF-LUV6													
3	UV9				BUV496	CD8	BP4-18								0.7	4.2			
4	UV10				BUV553	CD69	FM50								0.5	3.0		1	2.0
5	UV11				BUV635	CD44	1G1												
6	UV14				BUV661	VE2	B6								0.7	4.2		0.25	0.5
7	UV16				BUV737	CD38	1G6/CD38								1.3	7.8			
8	V1				BUV805	CD4	5S3											0.5	1.0
9	V3				BUV821	CD127	AO205												
10	V5				Pacific Blue	CD19	M5E2								2.0	12.0			
11	V7				BUV480	CD161	HP-3G10								2.0	12.0			
12	V10				BUV510	CD45RA	M120								0.7	4.2			
13	V11				BUV605	CD56	5.H11												
14	V13				BUV650	CCR7	GM43H7												
15	V14				BUV711	CD7	M170L											1	2.0
16	V15				BUV750	IFN γ	827												
17	B2				BUV786	CD86	11A9											1.5	3.0
18	B3				FTC/AF488	VA24/CD1d	8B11												
19	B4				Spartan blue 550	CD3	SK7												
20	B6				PE	INCG2D	1D11								1.2	7.2		0.5	1.0
21	B8				PE-CF594	CD28	M-A261											0.5	1.0
22	B10				PE-CF5	CD25	M-A251											0.3	1.0
23	B13				PerCP-CF5.5	TMF4	MbH1											0.5	1.0
24	R1				PerCP-770	PD1	PD3.1.3											0.5	1.0
25	R2				APC	CD16	3G8												
26	R4				AlexaFluor477	VA12/IRMB1	3C10												
27	R6				APC-FITC	CD107a	HA43												
28	R7				APC-FITC	CD27	N/A												
29	R8				APC-FITC	CD38	CD33												
And UNSTAINED CONTROLS (U)																			
Antibody Total									6.0	36								8.75	17.5
R10 Media									14.5	87								50	100
Pipette draw volume /sample									19.5									55.75	
Pipette draw volume /sample																			

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

Add Tetramin, 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
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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

805
20.5 p1
4.5 p1
4.5 p1

→ 11.8 p1 / polyalluvium

all in one

separate

PGS Perm

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	IFN γ					0.6	1.2
8	R670	Alexa 647	TNF α					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
Pipette draw volume / sample						19.5		19.5	



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