

April 7th, 2023

Cytokines afika?

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
Inf142 a-1	STRE	(3)	<6.75>			2	8.35 8.41	12.2	16.7		
Inf142 a-2	576F	(3)			clump	2	7.37	9.6	19.2 14.2		
NY062	Adult	(1)	20EG	08/12/21	clump	1	8.41	12.6	8.41		

spec pulled 10:14am
cord 10:20
stam for count @ 10:56am

Incubation @ 12:05 in top ↑ incubator rack PMA
yes spectral colcta

Aliquot spins @ 6:21pm

6:41 pm L/P

7:06pm L/O spin + se aliquot spin

<Stashed> @ 7:26pm cov & sc's

Spectral 37°C @ 7:30 → 8:00pm

Hot sc's @ 7:58pm → spin @ 8:01pm

Va 7.2/24/NK620 @ 8:11pm → 41pm ✓

Cold sc's @ 8:20pm → 8:50pm

Conventional sc's @ 8:25pm → 8:45pm

Cold cov & Spectral @ 20:56
→ 21:16 pm → 21:26pm

Sc's Fix Perm 200 pl (including PerCyt7)
@ 21:07pm → 17 → 27

Cov RBC lyse @ 6:18pm Spin @ 9:23pm ✓

Sc Fix Perm 1 & spectral final FBSE 21:36pm

	Conventional	Spectral
PermiWash 20 pl 11.25	0 2M 58.65	0 2M
50 pl	0 2M	0 2M
Amine 20 pl 11.25	0 2M	0 2M
50 pl	0 2M	0 2M
Adult ~20 M cord (2 Inf 142 vials) + 1 adult for sc's.	0 2M	0 2M

~~35.4M~~ 8.975
33.5M/4 8.375
No CD107a today? ... TBD

330 pl + 170 pl

360 pl + 140 pl

500 pl + 2 sc's
+ 1 sc (cov)

12 x 2 = 24 pl
x 500
6ml * 2 = 12 pl PMA

1ml + 10 pl SEB

1.5 ml + 6 pl bleach

1 - 0 Aqm
6K8 = 4800
→ 10 pl in 5 ml

1 - 0 NER
7 x 800 = 5600
3 pl in 750

20.5	
- 1.5 NK620	x 6 → 9
- 1.5 Va24	→ 9
- 1.2 Va.7	7.2
163 pl RBS	97.8

20.5
 9.25 μ l Abs + 50 μ l PermWash/Amine
 + 9.25 μ l
 11.25 μ l PermWash

Final sc PermWash @ 9:53 pm

Samples FixPerm @ 9:56 pm \rightarrow 06 \rightarrow 16 pm

CD56/cCR7 switched? adding BV650 cCR7 spike
 to supposedly cCR7 tube. check

sc spike intras @ 10:16 pm \rightarrow 56 pm

Sample 1st permWash @ 10:19 pm
 2nd @ 10:29 pm

~~Not spilling the 7.2 or the 24 just today~~ 0.3 μ l
 Yes all ILTs @ 0.3 μ l

Intracellular samples @ 11:06 pm

Done @ 12:05 am

LSR sample @ 3:18 (post sc acc)

~~210.8 11/12/88~~

April 7th, 2023

Saturday, April 8th

Cytokines afika?

LSR-II running bleach @ 12:19 pm

running water @ 12:28 pm

running contrast before 12:38 pm

running water @ 12:41 pm

Aurora on @ 12:22 pm

running water @ 12:34

↳ some pull in water, new tube @ 38 pm

QC @ 12:42 pm

QC's done @ 12:47 pm

Start Aurora @ 01:33 pm (Sc's out of order)

Sc's done @ 2:34 pm

Sample @ 2:40 pm

Myo2 Fucks (record)

(35 pl) only 646,000 events
|| || ||

730

300

555

580

610

525

415

520

540

640

650

580

LSR sample @ 3:18 pm today

39 - 38

Violetis off today,

(Fixing?)

Acquired 5 minutes on LSR (rightly 2 million cells)
~ 140 pl volume 0.4% PFA (~70 pl total)
and acquired on Spectral (some trial
& error on volume)

Direct comparison leftovers.

Initial Unmix²

8, 19, 161, 45RA, 56, 4a29, 16, 4a7.2

24 60

33 60

38 32

40 37

50 52

Ex-vivo gd-CK: PMA-ionomycin									
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	6	Intracellular 40min @RT	1
1	V450	BV421	PD1			2	12		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD27			1.5	9		
4	B530	Alexa 488	Vδ2			1.2	7.2		
5	B710	PerCPeF710	CD3			1	6		
6	Y590	PE	CD56			0.5	3		
	Y615	PE-Dazzle							
7	Y780	PE-Vio770	IFN γ	← Debris				0.6	0.6
8	R670	Alexa 647	TNF α					2	2
9	R780	APC-Fire750	CD45RO			2	12		
Antibody Total						8.2	49.2	2.6	2.6
PBS						12.3	73.8	17.9	17.9
Pipette draw volume / sample						19.5		19.5	

Spectral curve

PD1 standard for PE-Vio770
AF647 CD3

BV421 ✓

BV510 ✓

BV650 streamers ✓

F. to streamers ✓

PE ✓

AF ✓

Swapped PD1 for HCA-DR #1-surf3

PD1 crappy

Zenbe

LSR-II unstained
remnant cells in it

10% 8% 8%
4% 4% 4%

14% 60%
1%

4% 4% 4%
5% 5% 4%

14% 20%
7% 10%

11 | 9
5.2 | 4.4
9.5 4
9 4.1%



#	Filter	Single color (u)	Ref ctrl	Unmiring ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim	6	U/I 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @7°C	6	GoldStain 30min @4°C	6	RBC Lysa, then FxRperm	Spiled 30 min @RT	1
1	UV2				BUV395	CD21	SK11												
2	UV7				AF	AF-LIVE													
3	UV9				BUV496	CD8	RP4T8												
4	UV10				BUV563	CD69	FN50												
5	UV11				BUV615	CCR4	1G1												
6	UV14				BUV661	W2	B6												
7	UV16				BUV737	CCR3	1C6/CCR3												
8	UV1				BUV805	CD4	SK3												
9	UV3				BUV821	CD127	AD19D5												
10	UV5				Pacific Blue	CD14	M5E2												
11	UV7				Pacific Blue	CD19	S125C1												
12	UV10				BUV80	CD161	HP-3G10												
13	UV11				BUV805	CD36	5JH11												
14	UV13				BUV650	CCR7	GD34H7												
15	UV4				BU711	CD7	M-7T01												
16	UV5				BU750	IFN γ	B27												
17	B2				BU786	CCR6	11A8												
18	B3				ENC4/4488	V α 2/NC014	6811												
19	B4				Spark Blue 350	CD3	SK7												
20	B6				PE	INRG20	1011												
21	B8				PE-CF594	CD26	M-A261												
22	B10				PE-Cy5	CD25	M-A251												
23	B13				PMCP-Cy5.5	TM6a	MAB11												
24	R1				PE-4670	PD1	PD1.3.1.3												
25	R2				APC	CD16	3C8												
26	R4				Alexafluor647	V α 2/2HMR1	3C10												
27	R6				APC-R700	CD107a	H443												
28	R7				Zombie NIR	U/D	N/A												
29	R8				APC/Fire 750	CD27	G323												
					APC/Fire 810	CD38	H172												
Antibody Total								6.0	36										
R10 Media								14.5	87										
Pipette draw volume /sample								19.5											
Antibody Total								19.0	114	12.5	75.0	8.75	8.75						
Brilliant Stain								50	300	50.0	300	50	50						
Pipette draw volume /sample								66		59.5									
Antibody Total								1.6	12										
Brilliant Stain								9.6											
Pipette draw volume /sample																			
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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 PMACtrl 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, spin 1300 rpm, 8min

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 μ l 0.4% PFA-PBS
Cap tubes, wrap rack in foil, store at 4°C

Q.25 P.1

acid RNA stim \rightarrow Zembrine NER
PMR unstim \rightarrow 200/1
acid Zembrine PMR
PMR unstim \rightarrow 50/1 tubes

9
13 COL7a → Adult spectral reg PMA
16
11 ~~cord PMA~~
45 cord SEB green cord concentration
not

70% 1 ~ dose
3.2 ml/s cord
2x3 milk

4/7/2023

[illegible]

April 7th, 2023

Cytokines afika?

Leftover conventional mixing:

Full stain Conv Perm 60

BV421 mixing spur (supply in CD3)

Haotmg beads BV421

acBMC CD127

Spectral

- Zombie off
 - CCR7
 - VdL ← Cord CD2 issue
 - CD62
- fixing these

Spectral Salvage

CD3 gate set on NY062/SEB

CD107a & Zombie are off in full stain

Signature is off for Cord PMA conditions

Lineage + CD3+ CD62L

CD62L hi VdL CCR3 CCR7 IFN γ

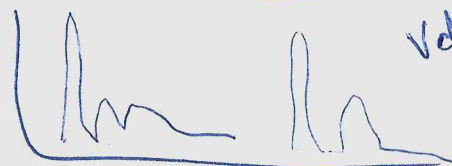
PMA Stimulating dying

All other Cord Zombies are Cord ctrl (only ILT-01 was a PMA

~ PMA NY062 didn't get properly stimulated ~ CD107a plateau split

~ Cord Zombie PMA weaker?

Let's replace w/ ILT-06's



VdL, Zombie, CD107a
may not apply due to label

CxCR3 - Zombie - LD107a

27 on Zombie but healthy ↓

+

W2 IFN γ

Live CD3

Running a PaeMAP:

"Admixtures w/ NKs by 2D gating"

TNF α issue

IFN γ +

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