

March 15th, 2023

2023_ILT_03

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
Inf148-3 a-4	57US HU Female	(2)	(14.78)			2	4.61		<u>9.22</u>		
148-3 a-3	57N4	(7)			(dead) Solid pellet left in blood	2	0.652				
Inf134-8 a-4	HEU-6 5702	(0)	(27.6)			2	2.75		5.5		
134-8 a-3	5704	(6)	(31.2)			2	2.77		5.54		
Inf159-5 a-3	HEU-hi 58FG	(3)				0.5	5.99		2.99		
159-5 a-3	58FJ	(4)				0.5	5.72		2.86		
ND050	Adult Norm	(0)	15E6	1/13/23		2	4.54				
Inf159-5 a-4	58FH	(8)	(16.16)			0.5	6.47		3.23		

10:39 am -> Thaw start

2nd spin @ 11:10 am

11:24 see DNA

Alta @ 12:35 pm -> 12:51 pm

Incubator @ 1:26 pm

Reagents finished @ 7:02 pm

Spin @ 7:38, 1300 rpm, 8 min
in 1 ml PBS

+ dead + dead zombie + new CD107a PMA
100 µl ND050

4:00 @ 7:56 pm -> 8:11 pm

37°C for samples @ 8:30 pm -> 9:00 pm
... at RT!

9:06 pm spin @ 1400 rpm 6 min

Tels @ 9:21 -> 10:01

Var 7.2 @ 9:34 pm / Spin @ 10:05 pm

4°C 10:19 -> 10:49 pm

RBC lysis @ 10:54

Final PBS spin @ 11:02 pm

Fix Perm @ 11:17 pm -> 27 -> 37 pm

1st wash @ 11:41 pm

2nd @ 11:54 pm

Intra @ 12:09 am

Final wash @ 12:54 am

Done @ 1:01 am

	Ly+M	con	1.5	2.5	3.0	1.0
Inf148	9.22	2	4.61	.325	.542	
Inf134	11.04	4	2.76	.543	.905	362 µl
Inf159	9.08	1.5	6.05	.248	.600	165 µl
ND050	9.08	2	4.54		.660	220 µl

	1.5	2.5	3.0	1.0
175 µl	0	0	0	0
0	0	0	0	0
253 µl	0	0	0	0
	0	0	0	0

440 Adult
x 2 x 2
880 µl + 4 µl
440 µl + 2 µl cHL
PMA

20.5	184.5
- 9	- 4
184.5 PBS	- 4
- 9.6 Var 7.2	176.5
- 12.0 Var 24	Peril
162.9	Wash

2010/01/02
land of 10/10/10

⑤

①

②

③

④

10/10/10 10/10/10

2/10

10/10/10
10/10/10

10/10/10
10/10/10

10/10/10

10/10/10

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Initial Unmix: w/ SC #2's

Swap in CD107~ 2 ND450 FMA into unmix set sch
Sen #2

ooo and about itooo. ~~drumroll~~ * you rolled ooo.

Small/big unmixing errors?

hard to see with detector staring

★ Good enough but will need fine tuning ★

1. The first part of the paper is devoted to a discussion of the general principles of the theory of the structure of the atom.

2. The second part of the paper is devoted to a discussion of the general principles of the theory of the structure of the atom.

3. The third part of the paper is devoted to a discussion of the general principles of the theory of the structure of the atom.

#	Filter	Single color (ul)	Ref ctrl	Umlinking ctrl name	Fluorescence	Marker	Clone	Vial Lot #	During stim III	14	U/D 15 min (RT)	Tetramer 40 min @ RT	Hoistain 30min @ 37C	14	Costain 30min @ 4C	14	RBC Lysate then FixPerm	Spiked 30 min @ RT	14
1	UV2				BUV395	CD95L	SK11								1.2	16.8			
2	UV7				AF	AT-UV6													
3	UV9				BUV496	CD8	RIA-78								0.7	9.8			
4	UV10				BUV653	CD69	FN50								0.5	7.0		1	14.0
5	UV11				BUV615	CCR4	1G1												
6	UV4				BUV661	Vβ2	86						2.0		0.7	9.8		0.5	7.0
7	UV16				BUV37	CCR3	1G6/CCR3								1.3	18.2			
8	UV1				BUV605	CD4	3K3												
9	UV3				BUV421	CD127	AD1905						1.5	21	2.0	28.0		0.5	7.0
10	UV5				Pacific Blue	CD19	M5E2						1.5	21	2.0	28.0			
11	V7				BUV480	CD181	HP-3G10						2	28	2.0	28.0			
12	V10				BUV510	CD45RA	HI100								0.7	9.8			
13	V11				BUV605	CD56	5.1H11												
14	V13				BUV50	CD7	GD3H7						1.0	14				1	14.0
15	V14				BUV711	CD7	M-1701						1	14					
16	V15				BUV750	IFRγ	B27												
17	B2				BUV786	CD86	11A9											1.5	21.0
18	B3				FFC/AF488	Vα2/CD14	6B11						1.5	21					
19	B4				Spark blue 550	CD3	5C7								1.2	16.8			
20	B6				PE	NG2D	1011						1.5	21				0.5	7.0
21	B8				PE-CF594	CD26	M-A261						1.2	16.8				0.5	7.0
22	B10				PE-CF55	CD25	M-A251						1.2	16.8					
23	B13				PE-CF670	TNFR	MA011											0.5	7.0
24	R1				APC	CD16	3G8								1.5	21.0		2.5	35.0
25	R2				Allophycocyanin	Vα2/CD161	3C10								0.7	9.8		0.5	7.0
26	R4				APC-700	CD107a	H4A3												
27	R6				Teconic V/R	U/D	N/A												
28	R7				APC/Fire 750	CD27	0323												
29	R8				APC/Fire 810	CD38	HT2												
Add UNSTAINED CONTROLS!!!																			
R10 Media									6.0	84		Antibody Total	19.0	266	12.5	175.0		9	126
Pipette draw volume / sample									14.5	203		Brilliant Stain	50	700	50.0	700		50	700
Pipette draw volume / sample									19.5			Pipette draw volume / sample	66		59.5			56	

64 51.5 54

Simplified Protocol

Thaw cells, DNase, count.

Collect, count, aliquot cells 2.30E6 Cells R10 / 5ml polypropylene tube
Bring volume up to 1 ml R10, add 2 ul PMA/CFI and CD107a
Cap and incubate at 37°C for 6 hours

Wash with 2 ml PBS, spin down 1300rpm 6min
800 ul of Live/Dead mix (1:2500) @ RT for 15min
Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 6min

Add Hoistain mix, incubate @ 37°C 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 Costain mix, incubate @ 4°C 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 @ 4°C 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

3/15/2023

[illegible]

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2:06 pm Acquisition start, event 7500

Review

1.5M

Inf 148 is ^{HSE} ~~data~~ component...

Chol tet → 2.3 million / 2.5

Dyn@ 3:07 pm : 17,000 cells event rate
(red flare)

200,000 / 400,000,000
100%

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