

DNA2
Rate March 17th, 2023

2023_ILT_04

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
Inf182	HU ♂										
a-3	SAS4	(3)	(7.2)			1.5	7.03	9.63	10.5		
a-4	SAS5	(7)	(7.8)			1.5	6.46	9.01	9.69		
Inf281	HEU-10										
a-1	SLMB	(4)	(13.2)			1.0	7.19	10.06	7.19		
a-2	SLMG	(6)	(15.9)			1.0	5.71	8.83	5.71		
Inf191	HEU-hi										
a-3	SLMB	(5)	(7.5)			2.0	4.88	7.91	9.76		
ND050	Adult Norm	(1)	15E6 (16)	1/13/23		1.5	6.47	8.66	9.7		
ND006	Adult sc's	(2)	15E6	1/20/23		1.5	9.00	11.0	13.5		

10:53am Thaw start

10:00 first cord thaw

11:12 first cord spin

11:35 2nd spin done

11:54am spin

Calc sheet setup @ 1:03 pm

1:36 pm cells aliquoted

Incubation @ 2:23 pm → 3:22 pm

ND006 PMA got 107A added
Sacrificed one ctrl's and added
2pl PMA + 2pl black...
TBD affect.

7:56 pm Ab prep starts

Spin @ 8:31 pm

Unaliquoted unstaineds
to the fridge @ 8:43 pm

Lpe 20:49 pm

Spin @ 9:09 pm

37°C @ 9:26 pm → 9:56 pm

Tab @ 10:05 pm

10:26 pm V07.2 → 10:56 pm Spin @ 11:04 pm

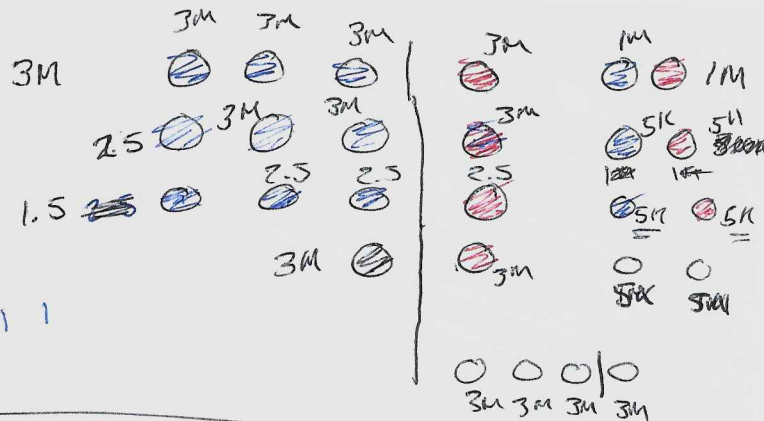
37 sec @ 10:00 → 11:30

4°C sc @ 11:26 → 11:56 pm

Samples 4°C @ 11:18 pm → 11:48 pm

	Lym	Conc
Inf182	20.19 / 3	6.73
Inf281	12.90 / 2	6.45
Inf191	9.76 / 2	4.88

6M left
(14M)



2.5	3M	450pl	+100	* 4	(1)
		430pl	+100	* 3	(1)
		440pl	+100	* 2	(1)
		570pl	+100	* 4	

69 pl
45 pl + 24 pl
70 pl

ND050 < 2 - PDI & cold)
ND006 for sc's PMA
* 1 < ctrl

37°C scs wash @ 11:30

Sample 42 → 11:48 pm

scs 42 → 11:56 pm

Final FBS spin @ 12:04 pm

FixPerm @ 12:23 pm → 33 → 43 pm
35 ✓

2nd Perm wash @ 1:10 am

Intra @ 1:40 am (scs away w/ 30 pl)
→ 2:20 am

Final wash @ 2:26 am

Done @ 2:41 am

$$\frac{2.75}{7} = \frac{3}{1}$$

$$2.75 = \frac{3}{6}$$

CD7 dumped?

Split CD45RA w/ CD14
after CD69 into CD14
original fck

No APC CD3...
PMA

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Sample	Total	Volume	Concentration	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF182	20.19	3	6.73	0.45	0.41	0.37	0.33	0.30	0.26	0.22	0.19	0.15	0.11	0.07
INF281	12.9	2	6.45	0.47	0.43	0.39	0.35	0.31	0.27	0.23	0.19	0.16	0.12	0.08
INF191	9.76	2	4.88	0.61	0.56	0.51	0.46	0.41	0.36	0.31	0.26	0.20	0.15	0.10
ND050	9.7	1.5	6.47	0.46	0.43	0.39	0.35	0.31	0.27	0.23	0.19	0.15	0.12	0.08
ND006	13.5	1.5	9.00	0.33	0.31	0.28	0.25	0.22	0.19	0.17	0.14	0.11	0.08	0.06

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF182	0.55	0.507	0.462	0.42	0.369	0.323	0.28	0.230	0.184	0.14	0.093
INF281	0.53	0.490	0.445	0.40	0.356	0.312	0.27	0.222	0.178	0.13	0.089
INF191	0.39	0.352	0.321	0.29	0.256	0.224	0.19	0.160	0.128	0.10	0.065
ND050	0.54	0.491	0.446	0.40	0.357	0.312	0.27	0.223	0.178	0.13	0.090
ND006	0.67	0.610	0.555	0.50	0.444	0.389	0.33	0.277	0.222	0.17	0.111

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF182	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF281	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
INF191	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
ND050	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33
ND006	2	1.83	1.66	1.5	1.32	1.16	1	0.83	0.66	0.5	0.33

Sample	3M	2.75M	2.5M	2.25M	2.0M	1.75M	1.5M	1.25M	1.0M	0.75M	0.5M
INF182	6	5.5	5	4.5	4	3.5	3	2.5	2	1.5	1
INF281	6	5.5	5	4.5	4	3.5	3	2.5	2	1.5	1
INF191	6	5.5	5	4.5	4	3.5	3	2.5	2	1.5	1
ND050	6	5.5	5	4.5	4	3.5	3	2.5	2	1.5	1
ND006	6	5.5	5	4.5	4	3.5	3	2.5	2	1.5	1

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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. In the second part, we shall consider the question of the influence of the external magnetic field on the structure of the atom.

3. The third part of the paper is devoted to a discussion of the question of the influence of the external electric field on the structure of the atom.

4. In the fourth part, we shall consider the question of the influence of the external magnetic field on the structure of the atom.

5. The fifth part of the paper is devoted to a discussion of the question of the influence of the external electric field on the structure of the atom.

6. In the sixth part, we shall consider the question of the influence of the external magnetic field on the structure of the atom.

7. The seventh part of the paper is devoted to a discussion of the question of the influence of the external electric field on the structure of the atom.

8. In the eighth part, we shall consider the question of the influence of the external magnetic field on the structure of the atom.

#	Filter	Single color (u)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial lot #	During stim(II)	14	L/D 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @37C	14	CD45Stain 30min @4C	14	RBC Lys. then Fix/Perm	Spiked 30 min @RT	14
1	UV2				BUV395	CD62L	SK11								1.2	16.8			
2	UV7				AF	AF-UV6													
3	UV9				BUV496	CD8	RP418								0.7	9.8			
4	UV10				BUV563	CD69	FN50								0.5	7.0		1	14.0
5	UV11				BUV661	CCR4	1G1						2.0	28					
6	UV4				BUV661	V82	B6								0.7	9.8		0.5	7.0
7	UV16				BUV737	CCR3	1G6/CCR3								1.3	18.2			
8	VA				BUV805	CD4	SK3						1.5	21				0.5	7.0
9	VA				BUV421	CD127	AD1905						1.5	21					
V3					Pacific Blue	CD14	M522								2.0	28.0			
10	V5				BUV480	CD19	S1291								2.0	28.0			
11	V7				BUV510	CD161	HP-3G10						2	28					
						CD45RA	H100								0.7	9.8			
12	V10				BUV605	CD56	5.1H11												
13	V11				BUV630	CCR7	GD3H17						1.0	14					
14	V13				BUV711	CD7	M-1701						1	14				1	14.0
15	V14				BUV750	IFN γ	B27												
16	V15				BUV786	CCR6	11A8											1.5	21.0
17	B2				HTC/AF488	Va24/NC01d	6B11						1.5	21					
18	B3				Spark blue 550	CD3	SK7												
19	B4				PE	NC62D	1D11								1.2	16.8		0.5	7.0
20	B6				PE-CF594	CD26	M-A261						1.5	21				0.5	7.0
21	B8				PE-CF594	CD25	M-A251						1.2	16.8				0.5	7.0
22	B10				PerCP-CF5.5	THFg	MbB11						1.2	16.8				0.5	7.0
23	B13				PE-40770	PD1	PD13.1.3											2.5	35.0
24	R1				APC	CD16	3C8								1.5	21.0		0.5	7.0
25	R2				AlexaFluor647	Va7.2/MAH1	3C10												
26	R4				APC-R700	CD107a	HA43												
27	R6				Zombie NIR	L/D	N/A												
28	R7				APC/Fire 750	CD27	CD32												
29	R8				APC/Fire 810	CD38	HT2												
Antibody Total									6.0	84	Antibody Total	19.0	266	12.5	175.0		9	126	
R10 Media									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		
And UNSTAINED CONTROLS III																			

Simplified protocol

Thaw cells, DNase, count.
Collect, count, aliquot cells 2.50E+6 cells R10 /5ml polypropylene tube
Bring volume upto 1 ml R10, add 2 ul PMA/CD and CD107a
Cap and incubate at 37°C for 6 hours

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

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

3/17/2023

[illegible]



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NDCCG ctrl+PNA experiment

under which card ~ Zone 2. 2023_ILT_04

Initial Unmixing: No Cords ... excess Inf182

* inserted NDCCG instances for group unmixing

