March 7th, 2023

1000 events: 1:100 2023_ILT_01

	Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	~~3~~2E+6	R10 ~~3E+6~~
1230μl 80μl	Inf0189 a-3	♂ HU RTL8		11.6μl (12)		bloody 1	2	5.06	6.76	10.12	395μl (5)	205μl
510μl 60μl	Inf161-8 a-3	♀ HEU-b 58KC		20.13μl (18)		not bloody 3 -clumps	2	5.01	8.09	10.02	399μl (5)	201μl
720μl 60μl	Inf180-9 a-3	♂ HEU-hi S9W8		20.7μl (15)		not bloody 2	2	3.85	5.27	7.7	519μl (4)	81μl
430μl 40μl	ND050	Adult Normal		21.2μl 15E6	1/13/23	1	2	4.64	6.35	9.28	431μl (5)	169μl
360μl 40μl	ND006	Adult SC's		23.0μl 15E6	1/20/23	2	2	3.55	4.93	7.1	563μl (3)	47μl

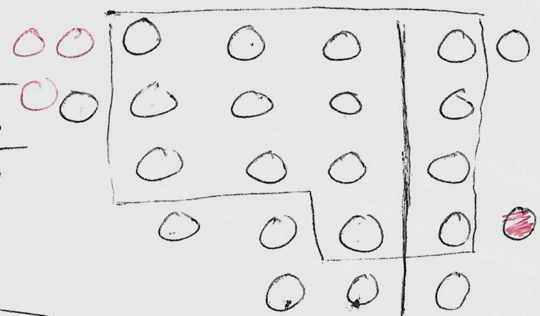
9:31 am cord thaw
9:49 am adult DNAse
Count start @ 10:13 AM
10:28 am count

[Guava was having issues after being left on for 4 days] and plate overspilled on guava... and pellets looked solid ... Guava's count is off?
→ Run @ 2E6 cells
(see what turns up on Aurora)
~3 E6 cells

11:41 am Incubation start, lower rack @ 37°C.
2 Million Lym/ml
1 ml
→ 5:41 pm out

[2μl/ml]
*
400μl R10 400
× 7.2 ×15
800 2000
28000 4000
2880μl & 14.4μl PMA

6000μl & 30μl golgi block



345
+130
-515
cord sc wash 2E8
200

Ab spin @ 4:34 pm
@ 5:31 pm

Sample spin @ 5:57 pm
4°C @ 6:18 pm
SC's 6:34
aliquoted → fridge

CCR7 → 14μl into intracellular, rest for sc...

4
15
× 8
12.0 mls
5μl in 12.5 mls

Hot samples @ 6:58 pm → 7:28 pm
(34) Spin @ 7:39 pm
SC's spin @ 7:09 pm
Hot SC's @ 7:35 pm → 8:05 pm
Cold SC's @ 8:00 → 8:30 pm spin @ 8:34 pm
Samples to 3°C @ 8:00 pm Pause!!!

1.2 20.5
×10 × 10
12μl 205μl
~~24~~ − 12
193μl

8:23 hot SC's paused pre-fix
8:29 tetramers → 9:10 pm
8:40 pm Va7.2/Va24!! (check background, 1.2 30 min RT vs 20 min RT?)
SC's FixPerm w/ 200μl @ 8:51 → 9:01 → 9:11 pm
Samples cold @ 9:32 pm → 10:02 pm
Samples post RBC lyse spin @ 10:11 pm
10:20 pm intrac SCs → 11:00 pm
10:25 FixPerm → 35 → 45

Going Forward
1.2 for abs
3μl 1:100 tet

Final SC spin & 2nd sample PennWash @ 11:04pm

SC's done @ 11:18 pm

Samples intake @ 11:27 (w/ appropriate Va 7.2/Va24 spikes)
⇒ 12:07pm

Done @ 12:24 am

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#	Filter	Single color (ul)	Ref ctrl	Unmixing ctrl name	Fluorochrome	Marker	Clone	Vial Lot #	During stim!!!	14	L/D 15 min (RT)	Tetramer 40 min @ RT	HotStain 30min @37C	14	ColdStain 30min @4C	14	RBC Lyse, then Fix/Perm	Spiked 30 min @RT	14
1	UV2	ND006 ctrl			BUV395	CD62L	SK11												
					AF	AF-UV6													
2	UV7	ND006 ctrl	/cord		BUV496	CD8	RPA-T8								1.2	16.8			
3	UV9	ND050	PMA		BUV563	CD69	FN50								0.7	9.8			
4	UV10	ND050	ctrl x2		BUV615	CCR4	1G1								0.5	7.0		1	14.0
5	UV11	ND050	ctrl x2		BUV661	Vδ2	B6						2.0	28					
6	U14	ND050	ctrl x2		BUV737	CXCR3	1C6/CXCR3								0.7	9.8		0.5	7.0
7	UV16	ND006	ctrl		BUV805	CD4	SK3								1.3	18.2			
8	V1	ND006	ctrl		BV421	CD127	A019D5						1.5	21				0.5	7.0
9	V3	ND006	ctrl		Pacific Blue	CD14	M5E2						1.5	21					
	V3	ND006	ctrl		Pacific Blue	CD19	SJ25C1								2.0	28.0			
10	V5	ND006	ctrl x2	/cord	BV480	CD161	HP-3G10								2.0	28.0			
11	V7	ND006	ctrl	/cord	BV510	CD45RA	HI100						2	28					
															0.7	9.8			
12	V10	ND006	ctrl x2		BV605	CD56	5.1H11												
13	V11	ND006	ctrl		BV650	CCR7	G043H7						1.0	14					
14	V13	ND006	ctrl		BV711	CD7	M-T701						1	14					
15	V14	ND050	PMA		BV750	IFNγ	B27						1	14				1	14.0
16	V15	ND006	ctrl x2		BV786	CCR6	11A9												
17	B2	ND050	ctrl x2		FITC/AF488	Va24/hCD1d	6B11						1.5	21				1.5	21.0
18	B3	ND006	ctrl		Spark blue 550	CD3	SK7												
19	B4	ND006	ctrl		PE	NKG2D	1D11								1.2	16.8		0.5	7.0
20	B6	ND006	ctrl		PE-CF594	CD26	M-A261						1.5	21				0.5	7.0
21	B8	~~ND050~~	PMA		PE-Cy5	CD25	M-A251						1.2	16.8					
22	B10	~~ND050~~	PMA		PerCP-Cy5.5	TNFα	MAb11						1.2	16.8					
23	B13	ND050	ctrl x2		PE-vio770	PD1	PD1.3.1.3											0.5	7.0
24	R1	ND050	ctrl		APC	CD16	3G8								1.5	21.0		2.5	35.0
25	R2	ND050	ctrl		AlexaFluor647	Va7.2/hMR1	3C10								0.7	9.8		0.5	7.0
26	R4	ND050	PMA		APC-R700	CD107a	H4A3		6.0	84									
27	R6	Cord			Zombie NIR	L/D	N/A				<1:2500>								
28	R7	ND050	ctrl		APC/Fire 750	CD27	O323						2	28					
29	R8	ND050	ctrl		APC/Fire 810	CD38	HIT2						1.6	22.4					
							Antibody Total		6.0	84	Antibody Total		19.0	266	12.5	175.0		9	126
And UNSTAINED CONTROLS !!!							R10 Media		14.5	203	Brilliant Stain		50	700	50.0	700		50	700
							Pippette draw volume /sample		19.5		Pippette draw volume /sample		66		59.5			56	

20.5
- 2.4
18.1

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

ND050x2

ND006 activated → PMA

- No CD3 or PD1 intra spike
- No surface CCR7 (½ dose added only in intra)

ILT & NK SFC Panel

3/7/2023

Spectrum	UV				Violet				Blue				Red			
373	UV1															
388	UV2	BUV395	[2]	CD62L												
428	UV3				V1	BV421	[4]	CD127								
443	UV4				V2											
458	UV5				V3	PacBlue	[1]	CD14/19								
473	UV6	AF			V4											
508					V5	BV480	[3]	CD161	B1							
514	UV7	BUV496	[2]	CD8												
525					V6				B2	FITC/AF488	[1.5]	Va24/hCD1d				
542	UV8				V7	BV510	[1.5]	CD45RA	B3	SparkBlue 550	[1]	CD3				
582	UV9	BUV563	[3]	CD69	V8	BV570			B4	PE	[4]	NKG2D				
598					V9				B5							
613	UV10	BUV615	[3]	CCR4	V10	BV605	[3]	CD56	B6	PE-CF594	[4]	CD26				
664	UV11	BUV661	[3]	Vd2	V11	BV650	[3.5]	CCR7	B7				R1	APC	[3.5]	CD16
679									B8	PE-Cy5	[4.5]	CD25	R2	AF647	[3.5]	Va7.2/hMR1
697	UV12				V12				B9	PerCP-Cy5.5	[2]	TNFa	R3			
717	UV13				V13	BV711	[4]	CD7	B10				R4	APC-R700	[3]	CD107a
738									B11				R5			
750	UV14	BUV737	[3]	CXCR3	V14	BV750	[2.5]	IFNg								
760									B12							
783	UV15				V15	BV786	[3]	CCR6	B13	Pe-Vio770	[3]	PD1	R6	Zombie NIR	[ ]	L/D
812	UV16	BUV805	[1]	CD4	V16				B14				R7	APC-Fire 750	[2]	CD27
													R8	APC-Fire 810	[3]	CD38

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Check for laser issues !!

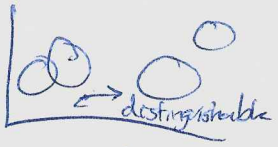
8:41: Template successfully set up !!

UV15/UV16
V51/V16
R6-R8
} Fails QC earlier

start @ 8:47 pm

CD62L 1:07:~~00~~ min & 250K cells
CD8 144K

Cord:



shape

CD69 works ✓ 150K events
(this was NDOSO?)

"CCR4 silver visible" (not brightest)
A lot of Vds NDOSO !!
decent ex CR3 stain

16t staining bit across all ... adult.
only slightly better cord

Δ intenst/shape to CD45RA cord/adult.
... pure Aft.
Strong IFNg

only 500 events for Va24 jackB (my suspicion why that first experiment failed so horridly)
good thing remembered Fite CD3

Solid PD1 expression
strong but faint CD16 signal.
Faint Va7.2 signal

¿Zombie Cordoooos ... unstained signature? wrap it in.

Sc's wrapped @ 9:59 pm

1:10:00 minutes

Samples @ 1001 pm

Info18

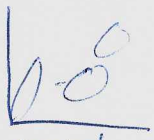
1,200,000 cells Info18 PMA 1,000,000
1,300,000 cells Info161 PMA (solid monocytes)
Info180 PMA

Last sample 10:33pm

Recorded 23 !!

¿Probably screen for dead-ish @ Guava & select for Zombie?

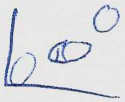
Info 161



Sc's 1 min

Samples 2 min

Info180



3 million cells/ml	1 ml	2 µl
2 million cells/ml	666/µl	1.33 µl
1 million cells/ml	333/µl	0.66 µl
500K cells	167/µl	0.33 µl
2.5 million cells/ml	833/µl	1.66 µl
1.5 million cells/ml	500/µl	1 µl

Samples_unstained @ 10:41 pm
~400K cells (~330K)

NDOO6_ctrl_sc Unstained_intracellular
NDOOE_PMA_sc Unstained_intracellular
NDO50_ctrl_sc Unstained_intracellular

Adult unstained ctrl PMA scs no intracellular stain

Shutdown @ 11:14 pm

Unmixing Ab Experiments

☆ NKG2D CD56 IFNγ TNFα CD107a ☆

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Initial Unmixing: unclear which cords used for what?

3Dead → 018

4 ~~Dead~~ Cord ctrl → 101 } ctrl

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