March 13th, 2023

2023_ILT_02

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
Inf070-7 a-3	HU ♀ RWKA	Box12A	44.8µl	③		2	1.77	2.41	3.54 / 4.94		
Inf374-4 a-7	HEU-106 SVHY	Box9B	19µl	④		2	3.85	5.45	7.7 / 10.9		
Inf039-2	HEU-4 ♀	Box2A	25µl	⑤	clump	2	3.64	6.56	7.28 / 13.12		
NDO50	Adult Normal,		20EG 25µl	1-19-23 ①		2	4.09	5.93	8.18 / 11.86		
NDOO6	Adult scs		15EG 17µl	1-20-23 ②		2	5.87	7.51	11.74 / 15.02		
Inf070			11.29µl	③		1	6.51	9.37	6.51/9.4		
Inf374a&b	SVHZ		8.46µl	④		1	7.49	11.0	7.49/11		
Inf039a2	RUTS		9.58µl	⑤		1	8.78	13.0	8.13/13		

60µl 430c
80µl 860c µl
60µl 840c µl
40µl 370c µl
40µl 420c µl
70 1440c µl
70 1640c µl
70 1760c µl

8:04 am Guava cleaning
8:18 am specimen pull
8:28 am Thaw
8:46 am Spin
9:03 am DVAsc
9:14 am stain for count
10:01 am 2nd thaw:
10:09 am spin
10:23 am DNAse
10:29 count stain
10:47 count done

11:43 at holding conc
11:56 am CD107a
12:09 pm Added PMA, → into incubator 6:09 pm

Combination CD107a
Ab prep @ 4:43 pm
Prepped @ 5:56 pm
Sample spin @ 6:19 pm
L/D @ 6:36 pm → 54 pm
spin @ 57
Scs aliquoted & spinning @ 6:43 pm
7:17 pm Hot samples → 47 pm
37°c Scs @ 7:26 pm → 54 pm
4°c Scs @ 7:41 pm → 8:11 pm
Samples spin 1400 rpm 6 min @ 7:52 pm

2nd set: Too concentrated (

3*3 = 9 + 1.5

Inf070	10.05	14.34	<2.5> (3 million)
Inf374	15.19	21.90	<3> (3 million left)
Inf039	16.06	26.20	<3> (4 million left)

NDO50 Va7.2 <3> Va72 <2.1>

NDOO6 <2.74>

IM (298)	conc @ 3ml	3M	2.5	1.5
147	Inf070 = 3.35	—	769µl	
229	Inf374 = 5.06	592µl		296
244	Inf039 = 4.35	689µl		
	NDO50	733µl		
	NDOO6	511µl		

20.5 x 9 = 184.5 PBS − 9.6 Va.72 − 12C Va24µl/0 = 162.9 PBS ... 21.6 aliq

20.5 x 9 = 184.5 PBS − 4.0 − 4.0 = 176.5 Perm wash

15 x .8 = 12.0 mls L/D NFR → 5µl in 12.5 mls

14 + 6 = 20 x .300 = 6.000 mls RBC lyse

Tcfr @ 8:09 pm → 8:49 pm
Va7.2 @ 8:21 pm
Scs Fix Perm @ 8:28 pm → 8:38 pm → 8:48 pm

Samples cold @ 9:08pm → 38 pm

1st sc Permwash @ 1400 rpm 6 min

2nd sc Permwash @ 9:20pm (1500 6 min

Most sc's 30µl PFA @ 9:41 pm

Rbc lyse samples 09:45pm

samples final FBS wash @ 9:50pm

sc mixes @ 9:57 → 10:37pm

Samples Fix Perm @ 10:05 → 15 → 25pm

Combined Permwash @ 10:44pm

<SH → <19.5>

Sc's done @ , w/ 30µl 0.4% PFA

Samples into intracellular @ 10:58, → 11:38 pm

Final spin 11:41 pm

Done @ 11:52 pm, cells resuspended in 50µl

<16 hrs>

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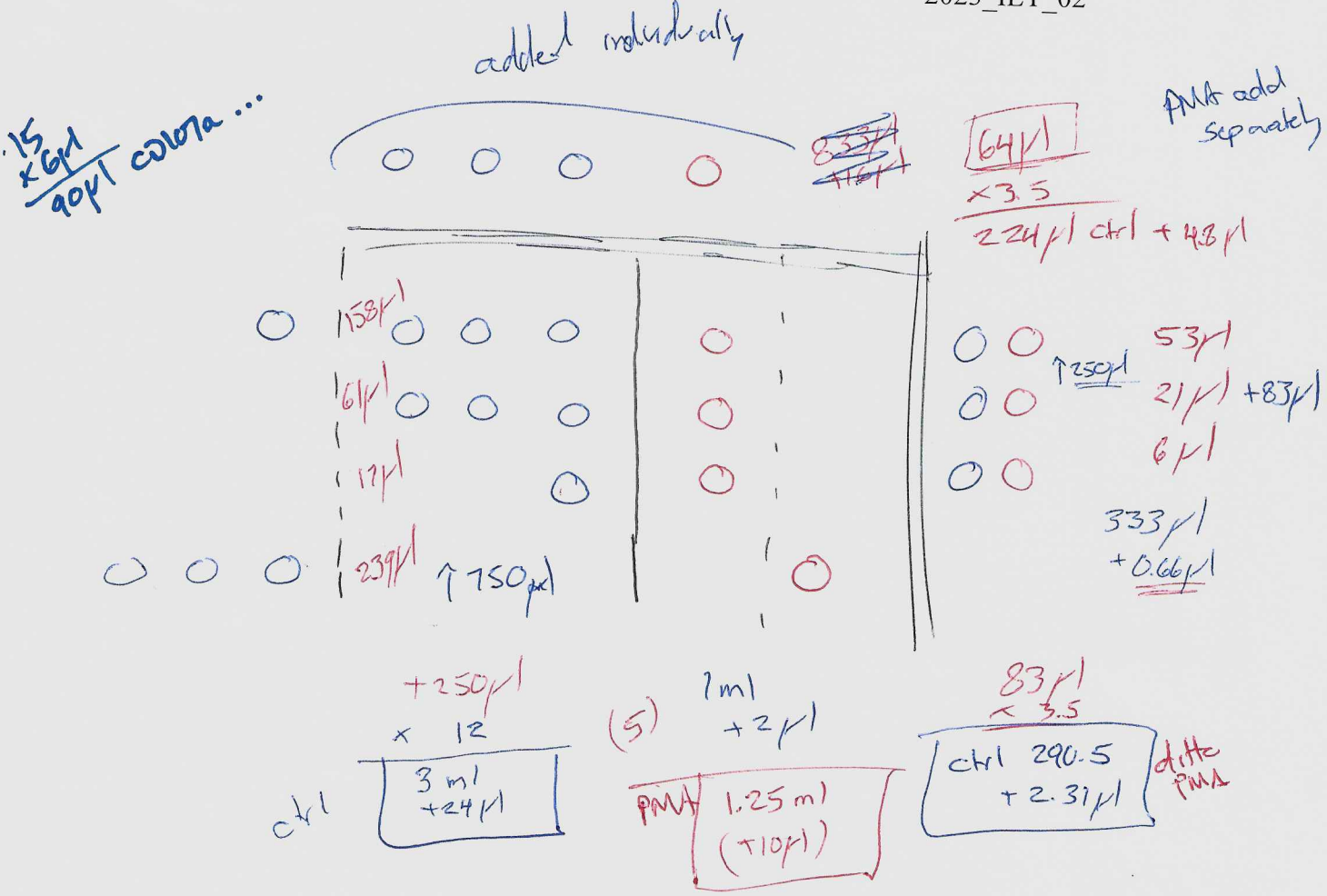

CD56 sc got 2µl instead 1µl
Cold 161 got a cold stain instead hot

March 13th, 2023 2023_ILT_01

#	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				BUV395	CD62L	SK11								1.2	16.8			
					AF	AF-UV6													
2	UV7				BUV496	CD8	RPA-T8												
3	UV9				BUV563	CD69	FN50								0.7	9.8			
4	UV10				BUV615	CCR4	1G1								0.5	7.0		1	14.0
5	UV11				BUV661	Vδ2	B6						2.0	28					
6	U14				BUV737	CXCR3	1C6/CXCR3								0.7	9.8		0.5	7.0
															1.3	18.2			
7	UV16				BUV805	CD4	SK3												
8	V1				BV421	CD127	A019D5						1.5	21				0.5	7.0
9	V3				Pacific Blue	CD14	M5E2						1.5	21					
	V3				Pacific Blue	CD19	SJ25C1								2.0	28.0			
10	V5				BV480	CD161	HP-3G10								2.0	28.0			
11	V7				BV510	CD45RA	HI100						2	28					
															0.7	9.8			
12	V10				BV605	CD56	5.1H11												
13	V11				BV650	CCR7	G043H7						1.0	14					
14	V13				BV711	CD7	M-T701						1	14				1	14.0
15	V14				BV750	IFNγ	B27						1	14					
16	V15				BV786	CCR6	11A9											1.5	21.0
17	B2				FITC/AF488	Va24/hCD1d	6B11						1.5	21					
18	B3				Spark blue 550	CD3	SK7												
19	B4				PE	NKG2D	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-Cy5	CD25	M-A251						1.2	16.8					
22	B10				PerCP-Cy5.5	TNFα	MAb11						1.2	16.8					
23	B13				PE-vio770	PD1	PD1.3.1.3											0.5	7.0
24	R1				APC	CD16	3G8								1.5	21.0		2.5	35.0
25	R2				AlexaFluor647	Va7.2/hMR1	3C10								0.7	9.8		0.5	7.0
26	R4				APC-R700	CD107a	H4A3		6.0	84									
27	R6				Zombie NIR	L/D	N/A				<1:2500>								
28	R7				APC/Fire 750	CD27	O323						2	28					
29	R8				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	

64 51.5 54

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

	37°C	4°C	cond
PMA x2	1	3½	
ctrl x2	3	4	2
ctrl x1	9	8	3

+ CD107a
+ Zombie cond
+ 1 cond sc ctrl

+1 NDOO6 SC ctrl
+1 NDOO6 SC PMA

14
+17
31

ILT & NK SFC Panel

3/13/2023

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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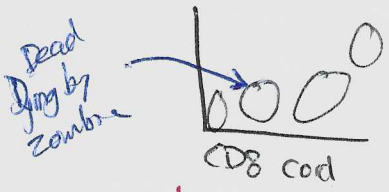
1:45 PM sc acquisition 30µl, 300,000 events

Sc's all ND006 or Inf374 ... -CD107a ND050 FMA

26.00 µl/min ~160,000 cells



no RBC lyse?
Yes Fixed Permed

CD69 ND006 ... TBD yes

BUV615 ... alternate stain? CD3/beads ...

... bulk pop negative ... enough events enough cells for positive

½ hour to get to cell (no unstaineds)

1 hour mark all sc's - 3 unstained (Chad add 2 ref, so essentially 1 hr !!)

~~There's~~ a bit more pronounced Blue peak AF for ND050 ctrl unstained ...

Sc's done @ 2:51 pm

2:56 unstained sample 1:56 ~ 850,000/1,000,000 recovery

Does incorporating in AF into sc signal make more difficult to separate? or does force separation @ the more distinctive sections?

~039 more dead cells, especially after FMA-ionomycin

Samples @ 3:12 pm

Done @ 3:52 pm (2:07:00 minutes)

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Intial unmixing: ND006 or Inf 374 scs

General AE extract

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