January 31st, 2023

HEU: Ex-vivo γδ CBMC panel #3 - 2023

	Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
70µl 1039[illegible]	Inf136-0 a-d	HU		14.6µl			2	4.52 ~~2.88~~	~~[illegible]~~.6.66			
60µl 500µl/l	Inf278-7 a-d	HEU-lo		38µl		Monocyte Heavy	1	2.15 ~~9.6~~	5.43 !!			
80µl 1540µl	Inf ~~587~~-0 a-d	HEU-hi		11.9µl		bbody	2.5	7.15	10.00			
40µl 8500µl/l	ND02 3 ~~[illegible]~~				10E7 4/4/2017		1	5.42	7.20			

Adult thaw @ 9:49 AM

cord all thawed @ 10:03 AM

10:17 AM DNAse

Count @ 10:29 AM
Stain

count guava @ 10:42 am

Culture in @ 11:48 AM

PMA (1.5M in 1M1 ~2µl respectively)
@ 12:05 pm → 6:05pm

Main Abs prepped @ 1:15 AM

1:20 pm (spin down)

1:32pm L/D → 1:47pm

~~&~~ Surface @ 14:08

2:36 RBC Lyse spin

streptavidin for P2
P1 PFAed
P4 6 holding

Strept wash @ 3:05pm

FixPerm @ 3:16 - 3:26 - 3:36

2nd Perm wash @ 3:44 pm

Intracellular @ 4:00pm

Done @16:53

6:07 pm

Cells spin post incubation ~~X~~

L/D @ 6:20pm

Spin @ 6:37pm

Surface @ 6:49pm

7:27 → 7:37 → 7:47

7:48 first permwash

Intracellular @ 8:03pm

Spin @ 8:42 pm

Done 8:50 pm

Co PM all P1	P4b	P7
		O
	O	O
OO O	O	O

800
×5
4.000 ml

3 ml - 3µl
[illegible]

4.5×2 = 9µl

9:24 ~~AM~~ PM → ~~Aurora~~ Larissa On
9:29 PM running control

↑ P4b to 730

4800 events Medium P4b

↑ high 10,600 events / sec @ 4:00 min medium

bottom at 2800 events

Inf 587 ~ 19,400 events (more activated?)

(LSR glitching @ 10:15pm)

1/31/23 10:01:49

→

Diva software
LSR crashed @ 10:21 pm (files fortunately saved)...
You Bastard!

10:26 pm (restart) Ctrl-Cll around 1300 events

wrong name.... 314 .. hell naaw...

★ Inf 314 [CD56 * PD1] officially screwed up, [dp] ★

Inf 278 ~ 16,700 events P2...

Glitched @ 10:58pm

FSC 680 for P1 Inf 587 ~ 14,000 events/sec

★ Turned on Avrom for Unstained. ← SSC glitch while collecting run on PeacockC

Done @ 11:27

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Ex-vivo Gd-P4b

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	2	Intracellular 40min @RT	2
1	V450	BV421	PD1			2	4		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD56			1.8	3.6		
4	B530	Alexa 488	Perforin					3	6
5	B710	PerCPeF710	CD3			1.5	3		
6	Y590	PE	GzmB					1.5	3
	Y615	PE-Dazzle							
7	Y780	PE-Vio770	NKG2A			0.6	1.2		
8	R670	APC	Vδ2			1	2		
9	R780	APC-Fire750	CD16			1	2		
					Antibody Total	7.9	15.8	4.5	9
					PBS	12.6	25.2	16	32
					Pippette draw volume / sample	19.5		19.5	

Ex-vivo Gd-P2

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	3	Streptavidin	Intracellular 40min @RT	3
1	V450	BV421	Vδ2			1	3			
2	V525	BV510	CD3			1.5	4.5			
3	V670	BV650	NKG2D			2	6	1.5		
4	B530	Alexa 488	Perforin						3	9
5	B710	PerCPVio700	CD56			1	3			
6	Y590	PE	PD1			1.5	4.5			
	Y615	PE-Dazzle								
7	Y780	PE-Vio770	NKG2A			0.6	1.8			
8	R670	APC	Vδ1			1	3			
9	R780	APC-Fire750	L/D Horizon		<1:1000>					
					Antibody Total	8.6	25.8	4.5	3	9
					PBS	11.9	35.7	57	17.5	52.5
					Pippette draw volume / sample	19.5		19.5	19.5	

Ex-vivo Gd-P1

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	1
1	V450	BV421	PD1			2	2
2	V525	BV510	L/D Aqua		<1:500>		
3	V670	BV650	CD16			1.5	1.5
4	B530	FITC	Vδ2			1.2	1.2
5	B710	PerCPeF710	CD25			2	2
6	Y590	PE	CD28			2	2
7	Y615	PE-Dazzle	CD27			1.5	1.5
8	Y780	PE-Vio770	CD3			0.5	0.5
9	R670	APC	Vδ1			1	1
10	R780	APC-Fire750	CD45RA			1.8	1.8
					Antibody Total	13.5	13.5
					PBS	7	7
					Pippette draw volume / sample	19.5	

Ex-vivo gd-CK: PMA-ionomycin

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	2	Intracellular 40min @RT	2
1	V450	BV421	PD1			2	4		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD27			1.5	3		
4	B530	Alexa 488	Vδ2			1.2	2.4		
5	B710	PerCPeF710	CD3			1	2		
6	Y590	PE	CD56			0.5	1		
	Y615	PE-Dazzle							
7	Y780	PE-Vio770	IFNγ					0.6	1.2
8	R670	Alexa 647	TNFα					2	4
9	R780	APC-Fire750	CD45RO			2	4		
					Antibody Total	8.2	16.4	2.6	5.2
					PBS	12.3	24.6	17.9	35.8
					Pippette draw volume / sample	19.5		19.5	

γδ T cell p1

1/28/2023

Spectrum		Violet			Blue				Red			
428	V1	BV421	[4]	PD1								
525	V6				B2	FITC	[1.5]	Vδ2				
542	V7	BV510	[1.5]	L/D Aqua	B3							
582	V8				B4	PE	[4]	CD28				
598	V9				B5							
613	V10				B6	PE-Dazzle594	[4]	CD27				
664	V11	BV650	[3.5]	CD16	B7				R1	APC	[3.5]	Vδ1
679					B8				R2	Alexa 647		
717	V13				B10	PerCP-eF710		CD25	R4			
783	V15				B13	Pe-Vio770	[3]	CD3	R7	APC-Fire 750	[2]	CD45RA
812	V16				B14				R8			

γδ T cell p2

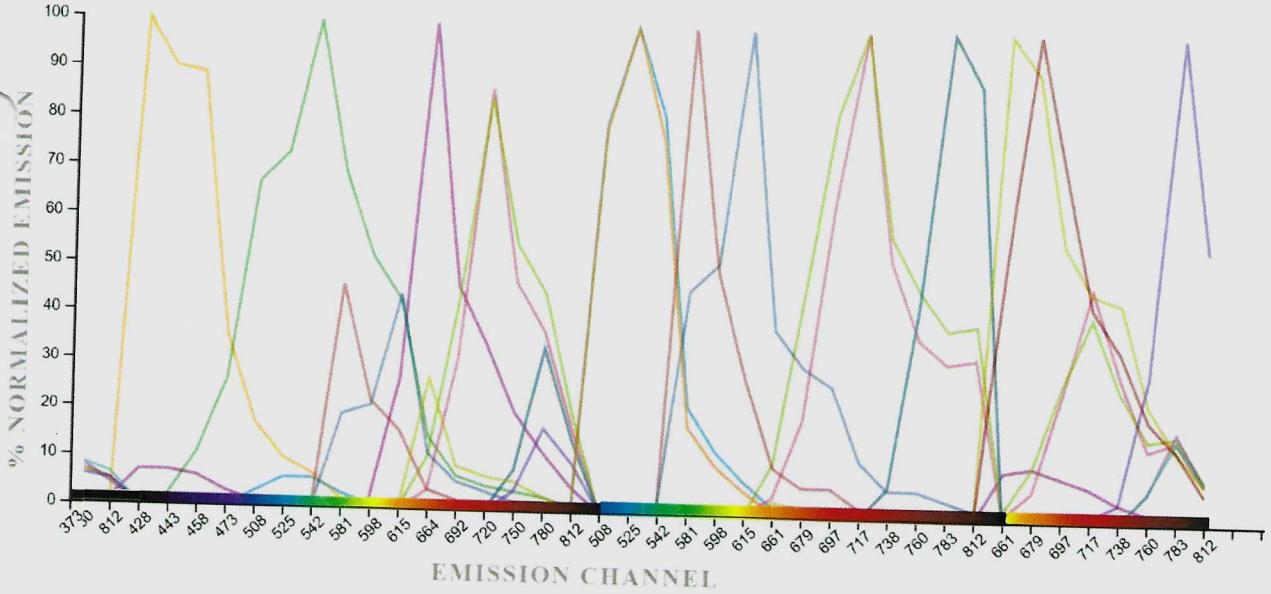
Spectrum		Violet			Blue				Red			
428	V1	BV421	[4]	Vδ2								
525	V6				B2	Alexa 488	[1.5]	Perforin				
542	V7	BV510	[1.5]	CD3	B3							
582	V8				B4	PE	[4]	PD1				
598	V9				B5							
613	V10				B6	PE-Dazzle594	[4]					
664	V11	BV650	[3.5]	NKG2D	B7				R1	APC	[3.5]	Vδ1
679					B8				R2	Alexa 647		
717	V13				B10	PerCP-Vio700		CD56	R4			
783	V15				B13	Pe-Vio770	[3]	NKG2A	R7	APC-Fire 750	[2]	L/D Horizon
812	V16				B14				R8			

γδ T cell p4b

Spectrum		Violet			Blue				Red			
428	V1	BV421	[4]	PD1								
525	V6				B2	Alexa 488	[1.5]	Perforin				
542	V7	BV510	[1.5]	L/D Aqua	B3							
582	V8				B4	PE	[4]	GrzmB				
598	V9				B5							
613	V10				B6	PE-Dazzle594	[4]					
664	V11	BV650	[3.5]	CD56	B7				R1	APC	[3.5]	Vδ2
679					B8				R2	Alexa 647		
717	V13				B10	PerCP-eF710		CD3	R4			
783	V15				B13	Pe-Vio770	[3]	NKG2A	R7	APC-Fire 750	[2]	CD16
812	V16				B14				R8			

γδ T cell CK

Spectrum		Violet			Blue				Red			
428	V1	BV421	[4]	PD1								
525	V6				B2	Alexa 488	[1.5]	Vδ2				
542	V7	BV510	[1.5]	L/D Aqua	B3							
582	V8				B4	PE	[4]	CD56				
598	V9				B5							
613	V10				B6	PE-Dazzle594	[4]					
664	V11	BV650	[3.5]	CD27	B7				R1	APC	[3.5]	
679					B8				R2	Alexa 647		TNFα
717	V13				B10	PerCP-eF710		CD3	R4			
783	V15				B13	Pe-Vio770	[3]	IFNγ	R7	APC-Fire 750	[2]	CD45RO
812	V16				B14				R8			



Similarity™ Indices

Configuration:4L 16UV-16V-14B-8R

	BV421	BV510	BV650	FITC	PerCP-eFluor 710	PerCP-Vio700	PE	PE-Dazzle594	PE-Vio770	PE-Cy7	APC	APC-Fire 750	Alexa Fluor 488	Alexa Fluor 647
BV421	1	0.17	0.1	0.01	0	0	0.01	0	0	0	0	0	0	0
BV510	0.17	1	0.16	0.06	0.03	0.03	0.23	0.18	0.01	0.01	0.02	0.01	0.01	0
BV650	0.1	0.16	1	0	0.3	0.33	0.06	0.17	0.04	0.04	0.33	0.05	0	0.16
FITC	0.01	0.06	0	1	0	0	0.17	0.12	0	0	0	0	1	0
PerCP-eFluor 710	0	0.03	0.3	0	1	0.99	0.04	0.18	0.36	0.37	0.25	0.17	0	0.22
PerCP-Vio700	0	0.03	0.33	0	0.99	1	0.05	0.22	0.4	0.4	0.25	0.17	0	0.21
PE	0.01	0.23	0.06	0.17	0.04	0.05	1	0.69	0.01	0.01	0.01	0	0.14	0
PE-Dazzle594	0	0.18	0.17	0.12	0.18	0.22	0.69	1	0.05	0.05	0.03	0	0.1	0
PE-Vio770	0	0.01	0.04	0	0.36	0.4	0.01	0.05	1	1	0.03	0.21	0	0.02
PE-Cy7	0	0.01	0.04	0	0.37	0.4	0.01	0.05	1	1	0.03	0.2	0	0.02
APC	0	0.02	0.33	0	0.25	0.25	0.01	0.03	0.03	0.03	1	0.17	0	0.93
APC-Fire 750	0	0.01	0.05	0	0.17	0.17	0	0	0.21	0.2	0.17	1	0	0.16
Alexa Fluor 488	0	0.01	0	1	0	0	0.14	0.1	0	0	0	0	1	0
Alexa Fluor 647	0	0	0.16	0	0.22	0.21	0	0	0.02	0.02	0.93	0.16	0	1
Complexity™ Index: 161.12														

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HEU: Ex-vivo γδ CBMC panel #3 - 2023

3 inf × 2 = 8 tubes — 12 tubes
1 adult × 2 + 8 tubes — + 8 tubes
16 tubes — 20 tubes

3 min
2 hr ½