February 2nd, 2023

HEU: Ex-vivo γδ CBMC panel #4 - 2023

	Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
1640 µl 8	Inf579-1 a-1	HU ♀ T60Q	②			rbc	2.5	3.29	6.13	8.225 / 15.325	5.1 ml	2.60 ml
700 µl 80 µl	Inf 702-1 a-1	TUX G HEU-lo ♀	③				2.5	4.42	6.19	11.05 / 15.475	5.16 ml	2.66 ml
700 µl 70 µl	Inf 438-5 a-2	TOR1 HEU-hi ♂	①				2	4.52	7.05	9.04 / 14.1	4.7 ml	2.7 ml
450 µl 30? µl	ND028			5E6 36 µl	4-12-2007		0.5	2.51	3.82	1.91	640 µl	140 µl
	ND033			5E6	07-21-2007		"	"	"	"	"	"

10:08 am prepped
10:15 am Adult spin
10:21 am
10:45 am DNAse
10:58 am stain for count?
BC6 @ 11:04 am
Count @ 11:17 am Into incubator?

→ 12:43 pm (spin down)

1.5 M
0.93 ← 579: 1.612 * 2.1 = 3.38
0.48 ← 0.70 ← 702: 2.141 * 2.6 = 5.56
0.78 ← 438: 1.923 * 1.7 = 3.27

* phu *

L/D @ 1:00 pm spin @ 1:18 pm
SC's @ 1:24 pm → 1:44 pm Rbc lyse ✓ spin @ 1:50 pm
Surface @ 1:31 pm → 1:51 pm rbc lyse @ 1:55 pm
(all reagents prepped @ 1:42 pm)
sc's done @ 2:05 pm in 70 µl 0.4% PFA-PBS

P- Strept @ 2:14 pm → 2:29 pm

pHb holding
P1 into 70 µl 0.4% PFA-PBS

Fix Perm @ 2:44 pm → 54 → 3:04 pm
1st perm wash @ 3:05 pm
2nd Perm wash @ 3:15 pm
3:26 pm Intracellular → 4:06 pm
Final Spin @ 4:07 pm

Done @ 16:17 pm

running Contrad @ 8:52

wake

↓ scatter 650

9:14 start

Moving all gates, setting middle highest peak

Samples @ 9:23 pm

P1 ↑ FSC to 700 // events 8300 on High

(maybe too high

< P4b issue > w/ graphs

P2 first @ 720

First P4b suffered

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Ex-vivo Gd-P4b

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	3	Intracellular 40min @RT	3
1	V450	BV421	PD1			2	6		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD56			1.8	5.4		
4	B530	Alexa 488	Perforin					3	9
5	B710	PerCPeF710	CD3			1.5	4.5		
6	Y590	PE	GzmB					1.5	4.5
	Y615	PE-Dazzle							
7	Y780	PE-Vio770	NKG2A			0.6	1.8		
8	R670	APC	Vδ2			1	3		
9	R780	APC-Fire750	CD16			1	3		
					Antibody Total	7.9	23.7	4.5	13.5
					PBS	12.6	37.8	16	48
					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	

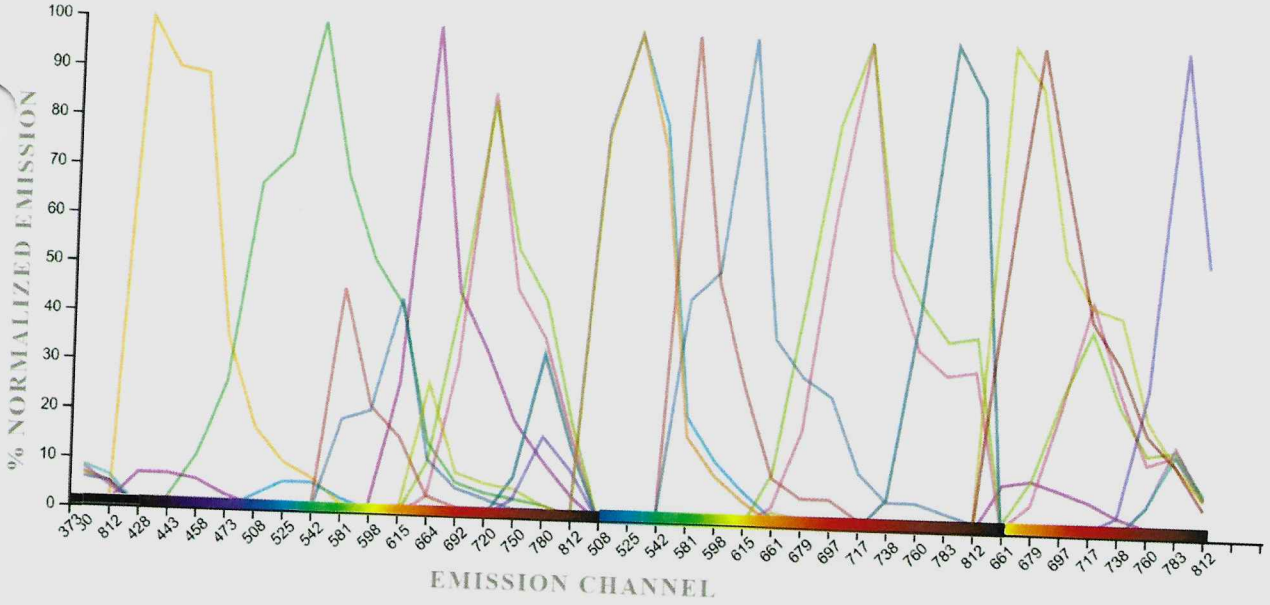
Ex-vivo Gd-P1

#	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	

ILT & NK SFC Panel

2/1/2023

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



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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1117-785-081