

30th
January 27th, 2023

HEU: Ex-vivo $\gamma\delta$ CBMC panel #2 - 2023

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	3E+6	.3E+6
664 Inf185 a2	HU SAXX		27 μ l		(2)	2	3.65	5.59	11.13 ^{4m}	3.72	1.72 ml
11 53 Inf281 Q-1	HEU-10 SLMB				(1)	1	6.94	10.06	10.06	3.33	2.33 ml
950 Inf314 a2	HEU-hi SNHC				(3)	2	5.15	7.34	14.72	4.90	2.90 ml
346 NY058			10 ⁷	12-16-15		2	4.56	6.45	12.9	4.30	2.30 ml

Adult NY058 @ 8:20 am

Thaw Inf @ 34, 38, 41,

9:09 stain

9:49 am BCG resuspended

Into Incubator @ 10:09 am

70, 60, 60, 40

Duke

[Cells were dumped blotted]

Cells (HEU-hi only) spinning @ 10:28 am

10:45 am LYO \rightarrow 11:00 AM

Abs spinning @ 10:49 am

11:22 pm Surface stain 4°C \rightarrow 11:42 pm

Mixes made 11:29 am

PDI/CD56
on PE \rightarrow P2 mix

Have been screened up

sc's m @ 11:36 am \rightarrow 56 pm

Spin @ 11:48 pm (post RBC lysis)

Cells fudge @ 11:58 pm [P2 sad face]

sc's post RBC spin @ 12:01 pm

stg P2 @ 12:13 (rooily mistake \rightarrow 28

12:42 \rightarrow 52 \rightarrow 1:02 pm

P1/P2 Perm @ 12:17 \rightarrow 27 \rightarrow 37

P1/unstained into 70 μ l ^{over} PFA PBS @ 1:02

[pub holding for P2]

Did you seriously just fix Perm the P1? Brch

1:17 pm Intracellular \rightarrow 01:57 pm

[Done @ 2:05 pm]

$$\text{HEU-hi} \quad \frac{14.72}{5.72} = 10.3 \quad [400 M]$$

$$\begin{aligned} 10.3 / 4.9 &= [2.10] \\ 1.5 M & \quad 4 \text{ m} \\ 9.12 / 4.3 &= [2.12] \\ \times 4 & \\ 8.12 & \\ - 3.34 & \\ \hline 4.78 & \\ 70? & \end{aligned}$$

$$\begin{aligned} 300K & \\ \times 11 & \\ \hline 3.3M & \end{aligned}$$

sc's 150

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Ex-vivo Gd-P4b

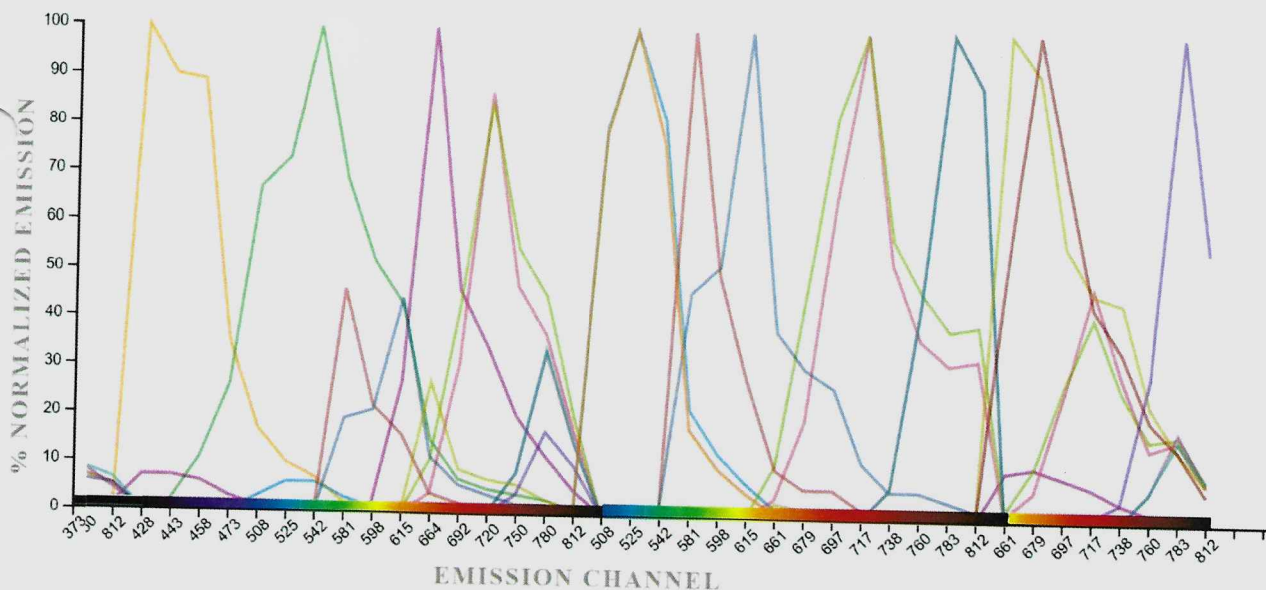
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	1	Intracellular 40min @RT	1
1	V450	BV421	PD1			2	2		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD56			1.8	1.8		
4	B530	Alexa 488	Perforin					3	3
5	B710	PerCPeF710	CD3			1.5	1.5		
6	Y590	PE	Gzmb					1.5	1.5
	Y615	PE-Dazzle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

Ex-vivo Gd-P2

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	1	Streptavidin	Intracellular 40min @RT	1
1	V450	BV421	Vδ2			1	1			
2	V525	BV510	CD3			1.5	1.5			
3	V670	BV650	NKG2D			2	2	1.5		
4	B530	Alexa 488	Perforin						3	3
5	B710	PerCPVio700	CD56			1	1			
6	Y590	PE	PD1			1.5	1.5			
	Y615	PE-Dazzle								
7	Y780	PE-Vio770	NKG2A			0.6	0.6			
8	R670	APC	Vδ1			1	1			
9	R780	APC-Fire750	L/D Horizon		<1:1000>					
Antibody Total						8.6	8.6	1.5	3	3
PBS						11.9	11.9	19	17.5	17.5
Pipette 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
Pipette draw volume / sample						19.5	



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

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	0	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	0.03	0.21	0	0.02	0.02
PE-Cy7	0	0.01	0.04	0	0.37	0.4	0.01	0.05	0.03	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	0	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

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]
679					B8				R2	Alexa 647	
717	V13				B10	PerCP-eF710		CD25	R4		
783	V15				B13	Pe-Vio770	[3]	CD3	R7	APC-Fire 750	[2]
812	V16				B14				R8		CD45RA

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]
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]
679					B8				R2	Alexa 647	
717	V13				B10	PerCP-eF710		CD3	R4		
783	V15				B13	Pe-Vio770	[3]	NKG2A	R7	APC-Fire 750	[2]
812	V16				B14				R8		CD16

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	
717	V13				B10	PerCP-eF710		CD3	R4		TNFα
783	V15				B13	Pe-Vio770	[3]	IFNγ	R7	APC-Fire 750	[2]
812	V16				B14				R8		CD45RO

Date		Description		Amount	
1917	Jan 1	Balance		100.00	
1917	Jan 15	Received from A. B. C.		50.00	
1917	Feb 1	Received from D. E. F.		25.00	
1917	Mar 1	Received from G. H. I.		75.00	
1917	Apr 1	Received from J. K. L.		100.00	
1917	May 1	Received from M. N. O.		150.00	
1917	Jun 1	Received from P. Q. R.		200.00	
1917	Jul 1	Received from S. T. U.		250.00	
1917	Aug 1	Received from V. W. X.		300.00	
1917	Sep 1	Received from Y. Z. A.		350.00	
1917	Oct 1	Received from B. C. D.		400.00	
1917	Nov 1	Received from E. F. G.		450.00	
1917	Dec 1	Received from H. I. J.		500.00	
1917	Total			2000.00	
1918	Jan 1	Balance		100.00	
1918	Jan 15	Received from K. L. M.		50.00	
1918	Feb 1	Received from N. O. P.		25.00	
1918	Mar 1	Received from Q. R. S.		75.00	
1918	Apr 1	Received from T. U. V.		100.00	
1918	May 1	Received from W. X. Y.		150.00	
1918	Jun 1	Received from Z. A. B.		200.00	
1918	Jul 1	Received from C. D. E.		250.00	
1918	Aug 1	Received from F. G. H.		300.00	
1918	Sep 1	Received from I. J. K.		350.00	
1918	Oct 1	Received from L. M. N.		400.00	
1918	Nov 1	Received from O. P. Q.		450.00	
1918	Dec 1	Received from R. S. T.		500.00	
1918	Total			2000.00	

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