

February 19th, 2023

HEU: D17  $\gamma\delta$  CBMC panel #4 - 2023

Specimen	Status	Location	Conc	Date	Volume	Ly 1:50	Ly+M	Total	1E+6	.66E+6	.5E+6
950gpl Inf579	IL2		25pl		4.5	4.3 2.15			465pl	307pl	232pl
888gpl	Zol		25pl		5	4.25 2.13				309pl	235pl
1200gpl	BCG		21pl		5	4.95 2.48				266pl	202pl
810gpl Inf702	IL2		30pl		4.5	3.61 1.8			555pl	367pl	278pl
800gpl	Zol		30pl		5.5	3.52 1.76				375pl	284pl
780gpl	BCG		25pl		5	4.16 2.08				317pl	240pl
840gpl Inf438	IL2		28pl		4.5	3.91 1.96			510pl	337pl	255pl
790gpl	Zol		20pl		5.5	4.98 2.49				265pl	200pl
760gpl	BCG				5	5.42 2.71				243pl	184pl
870gpl NDO28	Zol	bulldog? laser?				6.95 3.48				189pl	143pl

Reagents prepped @ 3:49 pm

start @ 3:56 pm

< 4:24 badl b. checker >

4:31 - count!

5:07 out for plate aliquots

5:18 spin

P2-4.4 spin @ 5:32 pm

Plate In @ 6:06 pm → 12:06 am

Sc's + Leftover spin @ 6:15 pm

r/o @ 6:24 pm

6:35 pm cells frozen away

spin @ 8:45 pm

Sc's @ 6:50 → 7:05 pm, spin @ 7:14 pm

Surface @ 7:01 pm → 7:21 pm

Spin @ 7:29 pm

Strip @ 7:42 pm → 57 pm 8 pm spin

P1 & sc's 1/50 pl @ 7:50 pm

Fix Perm 8:13 → 23 → 33

1st wash @ 8:36 pm End @ 8:48 pm

$$\begin{array}{r} 36 \\ \times 4 \\ \hline 144 \text{ mL} \\ + 22 \\ \hline 166 \text{ mL } 5\% \text{ FBS} \end{array}$$

$$\begin{array}{r} 36 \\ \times 4 \\ \hline 144 \text{ mL} \\ \text{Perm Wash} \end{array}$$

$$\begin{array}{r} 47 \\ \times 50 \\ \hline 2350 \text{ mL} \\ 0.9\% \text{ PFA} \end{array}$$

$$\begin{array}{r} 27 \\ \times 8 \\ \hline 216 \end{array}$$

Intra @ 9:01 pm → 9:41 pm

Done @ 10:02 pm

Due to worsening throat (and thesis next tomorrow)

12:09 am cd spinning

L/D @ 12:19 am → 34 am

spin @ 12:36

Surface @ 12:47 am → 1:07 am

1:10 spin

Fix Perm @ 1:22, 1:32, 1:42

First Perm Wash @ 1:43 am

2nd @ 1:52 am

L/D @ 2:02 am → 42 am

Done @ 2:55 am

2.  $\frac{1}{2}$  of the whole

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D17 Gd-P4b

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	9.5	Intracellular 40min @RT	9.5
1	V450	BV421	PD1*			2.5	23.8		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD56*			1.8	17.1		
4	B530	Alexa 488	Perforin					3.5	33.3
5	B710	PerCPeF710	CD3			1.5	14.3		
6									
7	Y780	PE-Vio770	NKG2A			0.6	5.7		
8	R670	APC	Vδ2			1.0	9.5		
9	R780	APC-Fire750	CD16			1.0	9.5		
Antibody Total						8.4	79.8	3.5	33.3
PBS						12.1	115.0	17.0	161.5
Pipette draw volume / sample						19.5		19.5	

D17 Gd-P2

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	9.5	Streptavidin	Intracellular 40min @RT	9.5
1	V450	BV421	Vδ2			1.0	9.5			
2	V525	BV510	CD3			1.5	14.3			
3	V670	BV650	NKG2D			2.0	19.0	1.5		
4	B530	Alexa 488	Perforin						3.5	33.3
5	B710	PerCPVio700	CD56			1.0	9.5			
6	Y590	PE	PD1			1.5	14.3			
7	Y780	PE-Vio770	NKG2A			0.6	5.7			
8	R670	APC	Vδ1			1.0	9.5			
9	R780	APC-Fire750	L/D Horizon		<1:1000>					
Antibody Total						8.6	81.7	14.3	3.5	33.3
PBS						11.9	113.1	180.5	17.0	161.5
Pipette draw volume / sample						19.5		19.5	19.5	

D17 Gd-P1

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	9.5
1	V450	BV421	PD1			2.5	23.8
2	V525	BV510	L/D Aqua		<1:500>		
3	V670	BV650	CD16			1.5	14.3
4	B530	FITC	Vδ2			1.2	11.4
5	B710	PerCPeF710	CD25			2.0	19.0
6	Y590	PE	CD28			2.5	23.8
7	Y615	PE-Dazzle	CD27			1.5	14.3
8	Y780	PE-Vio770	CD3			0.5	4.8
9	R670	APC	Vδ1			1.0	9.5
10	R780	APC-Fire750	CD45RA			1.8	17.1
Antibody Total						14.5	137.8
PBS						6.0	57.0
Pipette draw volume / sample						19.5	

D17 gd-CK: α-γδTCR

#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	9.5	Intracellular 40min @RT	9.5
1	V450	BV421	PD1			2.5	23.8		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD56			1.5	14.3		
4	B530	Alexa 488	CD107a						
5	B710	PerCPeF710	TNFα					<3 uL>	
6	Y590	PE	Vδ2			1.0	9.5	2.5	23.8
7	Y780	PE-Vio770	IFNγ						
8	R670	Alexa 647	CD27			2.0	19.0	1.1	10.5
9	R780	APC-Fire750	CD45RO			1.8	17.1		
Antibody Total						8.8	83.6	3.6	34.2
PBS						11.7	111.2	16.9	160.6
Pipette draw volume / sample						19.5		19.5	





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Feb 21st: 6:38pm (acquisition) (little under 48 hrs for P2/P4b)

Fsc @ 7:30 for P1  $\checkmark$  P2

P4b @ 8:09 pm

cu @ 8:45 pm

Dave @ 9:18 pm



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