August 67, 2021

HEU: P2 + P4b γδ Cord Optimization #2

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+M	Total	1.5E+6	2E+6
300µl NY054	Adult		10x7	11/12/15	P4b tests	3 ml	3.56 E4	4.68E4	10.68	420µl	9q1 scs
350µl CQ1047	Cord					3 ml	1.30 E4	1.51E4	4.53		1.54mls

June	Ex vivo Gd-p2	Surface µL	x	Intra µL	x
BV421	VD2	1			
BV510	CD3	1.5			
BV650	NKG2D	2.0			
Alexa 488 (FITC)	dg9	-		3	
PerCPeF710	CD56	1			
PE	PD1	1.5			
PE Dazzle					
PE Vio770	NKG2A	0.6			
APC	VD1	1.0			
APC Fire750	Horizon L/D	1:1000			
20.5 µl/rxn;	PBS	~~13.4~~ 11.9		17.5	

June	Ex vivo Gd-p2	Surface µL	x	Intra µL	x
BV421	VD2	1			
BV510	CD3	1.5			
BV650	NKG2D	2 ~~2 2~~			
	Streptavidin	1.5			
Alexa 488 (FITC)	dg9	-		3	
PerCPeF710	CD56	1.0			
PE	PD1	1.5			
PE Dazzle					
PE Vio770	NKG2A	0.6			
APC	VD1	1.0			
APC Fire750	Horizon L/D	1:1000			
20.5 µl/rxn;	PBS	11.9		17.5	

20.5 - 8.1 = 11.9 12H

Thaw start @ 10:08 am
Spin @ 10:18 60µl DNAse (not very many cord...)
Stain @ 10:37

*Guava being hella uncooperative this fxn)
Very few monocytes in this cord blood sample.

3µl - 3ml Horizon
4x8 = 3.2 3.5ml - 7µl Aqua

Streptavidin → before or after RBC lysis
2bkm + 1.5µl
* for this round *

Plating tubes @ 11:10 am
Made new scs. to be safe

L/D @ 11:42
Scs @ 11:54 → 12:19 → Done @ 12:35pm

Surface @ 12:19 RBC lyse 12:42 → 46

Biotin @ 12:59 pm +15 → 1:14 ✓
Fix Perm @ 1:02 → 1:12 → 1:22
strep FixPerm @ 1:26 pm → 1:36 → 1:46
Intracellular @ 1:43 pm → 2:23 pm
@ 2:03 pm → 2:43 pm

Strep 14ml 1hr @ RT
wash w/ 2ml 5% FBS-PBS
(Δ vs 1ml of PBS?)

Done @ 14:56 pm

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Testing on an adult

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Hao-Ting & November	Ex vivo Gd-p4b	Surface µL	x	Intra µL	x
BV421	PD1 ✓	2			
BV510	Aqua L/D	1:500			
BV650					
Alexa 488 (FITC)	Perforin (dG9)	-	-	2	
PerCPeF710	CD3	1.5			
PE	GZMb	-	-	1.5	
PE Dazzle					
PE Vio770	NKG2A	0.6			
APC	CD56	1.3			
APC Fire750	VD2	1.2			
20.5 µl/rxn;	PBS	12		17.0	

(13.9)

Switchup	Ex vivo Gd-p4b	Surface µL	x	Intra µL	x
BV421	PD1	2			
BV510	Aqua L/D	1:500			
BV650					
Alexa 488 (FITC)	Perforin (dG9)	-	-	2	
PerCPeF710	CD3	1.5			
PE	GZMb	-	-	1.5	
PE Dazzle					
PE Vio770	NKG2A	0.6			
APC	VD2	1.3			
APC Fire750	CD56	1.2			
20.5 µl/rxn;	PBS	12		17.0	

13.1

Master Mix

Current Version	Ex vivo Gd-p4b	Surface µL	x	Intra µL	x55
BV421	PD1 ✓	2			
BV510					
BV650	CD56	1.8			
Alexa 488 (FITC)	Perforin (dG9) ✓	-	-	3	165
PerCPeF710	CD3 ✓	1.5			
PE	GZMb ✓	-	-	1.5	82.5
PE Dazzle					
PE Vio770	NKG2A ✓	0.6			
APC	Vδ2	1			
APC Fire750	Horizon L/D	1:1000			
20.5 µl/rxn;	PBS	13.6		16	88

Test Aqua	Ex vivo Gd-p4b	Surface µL	x	Intra µL	x
BV421	PD1	2			
BV510	Aqua L/D	1:500			
BV650	CD56	1.8			
Alexa 488 (FITC)	Perforin (dG9)	-	-	3	
PerCPeF710	CD3	1.5			
PE	GZMb	-	-	1.5	
PE Dazzle					
PE Vio770	NKG2A	0.6			
APC	Vδ2	1.0			
APC Fire750					
20.5 µl/rxn;	PBS	13.6		16	

Test Aqua + CD16	Ex vivo Gd-p4b	Surface µL	x	Intra µL	x
BV421	PD1	2			
BV510	Aqua L/D	1:500			
BV650	CD56	1.8			
Alexa 488 (FITC)	Perforin (dG9)	-	-	3	
PerCPeF710	CD3	1.5			
PE	GZMb	-	-	1.5	
PE Dazzle					
PE Vio770	NKG2A	0.6			
APC	Vδ2	1			
APC Fire750	CD16	1			
20.5 µl/rxn;	PBS	12.6		16	

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HEU: Innate-like T cell Panels
Tetramer Panels

	Ex vivo MAIT-p1a	Surface µL	x
V450 BV421	Va7.2	2	
V525 BV510	Aqua L/D	1:500	
V670 BV650			
B530 FITC	CD4	(1.5)	
B710 PerCPeF710	CD45RA	2	
Y590 PE	CD161	0.5	
Y615 PE Dazzle			
Y780 PE Vio770	CD3	0.5	
R670 APC	CCR6	(2.5)	
R780 APCFire750	CD8	(1.2)	
20.5 µl/rxn;	PBS	10.3	

Concurrent Staining (20 minutes, 4*C)

(1.1000)

Tetramer Test A1	Ex vivo MAIT-tet	Surface µL	x
V450 BV421	CD4	1.5	
V525 BV510	Aqua L/D	1:500	
V670 BV650			
B530 Alexa488 (FITC)	6FP/ 5OP-RU		
B710 PerCPeF710	CD45RA	2	
Y590 PE	CD161	0.5	
Y615 PE Dazzle			
Y780 PE Vio770	CD3	0.5	
R670 APC	(CCR6)		
R780 APCFire750	CD8	1.2	
20.5 µl/rxn;	PBS		

Separate Staining (20 minutes tetramer RT, 20 minutes 4*C)

(1:1000)

Tetramer Test A2	Ex vivo MAIT-tet	Surface µL	x
V450 BV421	CD4	1.5	
V525 BV510	Aqua L/D	1:500	
V670 BV650			
B530 Alexa488 (FITC)	6FP/ 5OP-RU		
B710 PerCPeF710	CD45RA	2	
Y590 PE	CD161	0.5	
Y615 PE Dazzle			
Y780 PE Vio770	CD3	0.5	
R670 APC	(CCR6)		
R780 APCFire750	CD8	1.2	
20.5 µl/rxn;	PBS		

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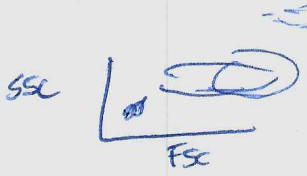
Flow panels set up @ 10:33 am ✓
- adjusted gate to cut out slimpy curve on all....



running @ 124 evts/sec

Run start @ 10:43 am ✓ ↑ 580 FSC

Biotin'd NR620 does load better.... pd1 balls squished? cD3-

☆ clot ☆ 11:06 am ✓
clot resolved @ 11:11?

☆ water tube had ten cells ☆

¿ @ stop acquire, how long tube to fall back in?
- Does this happen?

B running dirty 3500 evts/sec ☆ BD heavy non specifics ☆

Done @ 11:55 am

Culture Set up: D0 D10 D12

1.5 EG L+M/ml