

September 9th, 2021

HEU: P4b $\gamma\delta$ Cord Expansion D¹⁷

Specimen	Status	<- ml	<- Conc	Tasks	Volume	Ly	Total	.5E+6	.3 E+6
NXXXX	Adult				3.8	3.3E6	12.54	150 μ l	9 μ l
CQ1070	D17 cord				5.5	3.24E6	17.82	154 μ l	93 μ l

Current Version	Ex vivo Gd-p4b	Surface μ L x 12	Intra μ L x 18
BV421	PD1	2	24
BV510			
BV650	CD56	1.8	21.6
Alexa 488 (FITC)	Perforin (dG9)	-	3
PerCPeF710	CD3	1.5	18
PE	GZMb	-	1.5
PE Dazzle			
PE Vio770	NKG2A	0.6	7.2
APC	V82	1	12
APC Fire750	Horizon L/D	1:1000	
20.5 μ l/rxn;	PBS	13.6	163.2

Test Aqua	Ex vivo Gd-p4b	Surface μ L x 2.5	Intra μ L x
BV421	PD1	2	5
BV510	Aqua L/D	1:500	
BV650	CD56	1.8	4.5
Alexa 488 (FITC)	Perforin (dG9)	-	3
PerCPeF710	CD3	1.5	3.8
PE	GZMb	-	1.5
PE Dazzle			
PE Vio770	NKG2A	0.6	1.5
APC	V82	1.0	2.5
APC Fire750			
20.5 μ l/rxn;	PBS	13.6	34

Not enough $\gamma\delta$ Horizon $\gamma\delta$ cord? Seeded @ 3:04 in @ 3:07
 1:40 reagents, prepped
 1:55 RBS wash
 - 58 spin
 - 2:05 spin
 2:15 417
 SCS cyto prep
 @ 2:26 pm
 Spin 2ml
 FBS @ 2:32
 Surface
 SCS in
 @ 2:44 pm

Test Aqua + CD16	Ex vivo Gd-p4b	Surface μ L x 2.5	Intra μ L x
BV421	PD1	2	5
BV510	Aqua L/D	1:500	
BV650	CD56	1.8	4.5
Alexa 488 (FITC)	Perforin (dG9)	-	3
PerCPeF710	CD3	1.5	3.8
PE	GZMb	-	1.5
PE Dazzle			
PE Vio770	NKG2A	0.6	1.5
APC	V82	1	2.5
APC Fire750	CD16	1	2.3
20.5 μ l/rxn;	PBS	12.6	31.5

27 fixative
 @ 3:22 pm
 3:24
 3:27
 3:31
 Wait spin on step 2
 by 3:30
 1st spin
 wait @
 3:50 pm
 SCS fixative
 @ 3:55
 4:05
 4:15
 SCS buffer
 done @ 4:20

Intra @ 4:14 pm \rightarrow 4:54 pm
 SCS fixed 1st wash @ 4:18 pm
 2nd @ 4:31
 P₂ intra @ 4:41 pm \rightarrow 5:21 pm
 SCS done @ 4:49 pm
 Main done @ 5:10 pm
 T₂ 20 led @ 5:16 pm

10
 30 min 4x15 = 1hr
 18 min 6
 6
 22 x 3 = 66
 44 x 3 = 132
 98 mls
 327
 44 x 6 = 264
 264 x 2 = 528
 528 x 2 = 1056
 1056 x 2 = 2112
 2112 x 2 = 4224
 4224 x 2 = 8448
 8448 x 2 = 16896
 16896 x 2 = 33792
 33792 x 2 = 67584
 67584 x 2 = 135168
 135168 x 2 = 270336
 270336 x 2 = 540672
 540672 x 2 = 1081344
 1081344 x 2 = 2162688
 2162688 x 2 = 4325376
 4325376 x 2 = 8650752
 8650752 x 2 = 17301504
 17301504 x 2 = 34603008
 34603008 x 2 = 69206016
 69206016 x 2 = 138412032
 138412032 x 2 = 276824064
 276824064 x 2 = 553648128
 553648128 x 2 = 1107296256
 1107296256 x 2 = 2214592512
 2214592512 x 2 = 4429185024
 4429185024 x 2 = 8858370048
 8858370048 x 2 = 17716740096
 17716740096 x 2 = 35433480192
 35433480192 x 2 = 70866960384
 70866960384 x 2 = 141733920768
 141733920768 x 2 = 283467841536
 283467841536 x 2 = 566935683072
 566935683072 x 2 = 1133871366144
 1133871366144 x 2 = 2267742732288
 2267742732288 x 2 = 4535485464576
 4535485464576 x 2 = 9070970929152
 9070970929152 x 2 = 18141941858304
 18141941858304 x 2 = 36283883716608
 36283883716608 x 2 = 72567767433216
 72567767433216 x 2 = 145135534866432
 145135534866432 x 2 = 290271069732864
 290271069732864 x 2 = 580542139465728
 580542139465728 x 2 = 1161084278931456
 1161084278931456 x 2 = 2322168557862912
 2322168557862912 x 2 = 4644337115725824
 4644337115725824 x 2 = 9288674231451648
 9288674231451648 x 2 = 18577348462903296
 18577348462903296 x 2 = 37154696925806592
 37154696925806592 x 2 = 74309393851613184
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 304371277216207601664 x 2 = 608742554432415203328
 608742554432415203328 x 2 = 1217485108864830406656
 1217485108864830406656 x 2 = 2434970217729660813312
 2434970217729660813312 x 2 = 4869940435459321626624
 4869940435459321626624 x 2 = 9739880870918643253248
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September , 2021

HEU: P4b $\gamma\delta$ Cord Expansion D10

Hao-Ting & November	Ex vivo Gd-p4b	Surface $\mu\text{L} \times 2.5$		Intra $\mu\text{L} \times 2$	
BV421	PD1	2	5		
BV510	Aqua L/D	1:500			
BV650					
Alexa 488 (FITC)	Perforin (dG9)	-	-	2	
PerCPeF710	CD3	1.5	3.8		
PE	GZMb	-	-	1.5	
PE Dazzle					
PE Vio770	NKG2A	0.6	1.5		
APC	CD56	1.3	3.25		
APC Fire750	VD2	1.2	3		
20.5 $\mu\text{L}/\text{rxn}$	PBS	12	34.8	17.0	

13.9

Failed to remove

Voltage \approx compensation (values or ratio?)
630 FSC 580

RG 70 300 530
R7 80 300 540
BS 30 580
B7 10 530
VH 50 445
VS 25 500
V6 70 550
Y5 90 570
Y6 15 640
Y7 80 515

FSC CQ1070 A 640
RB70 570
on these FSC gate is off
PerCP 560
B256 CD56 520

Div2: Gated sc's w/ FSC property

- moved voltages a good chunk for SC Add, ^{comp} gates as needed
- for subsequent if comparable, voltage will need to be fixed

\neq apply for CD56 tubes just A, i

All using Addt not fixed.

- some AF for these samples in SC

- the point being test vs Δ compensation controls.
- so fingers crossed on 06 voltage

QD3 CQA:

Question of how far have I adjusted/squished things over last month?

Seems mostly cleaner as far as Diva compensation,

result of my FSC gating on SCs?

CD56: band of CD56, I expanded my compensation gate to grab all?
adjusted down to 520 (bright under 10⁵) and matched gate, overwrote prev ✓
* FSC/SSC gate was ^{just} off ...
- was able to drag once acquired ... ✓ for now?

Brian's charger hits my phone

Q1070 A: running normally \approx CD56 where should be at. \rightarrow next versions should be copy of CD3 file not this one.

CD56 BDE: ^{FSC} SSC gate on CD56 \rightarrow some off despite setting gate unstained vs stained?

CD56 PerCP P2 \rightarrow also way Δ FSC profile vs CD3 stained.
really off @10 FSC unstained vs 580 after fix

630 FSC

This ^{sub-} Experiment was done @



#2:

Adjust Fixed SSC:

SSC much more spread out after fixing

270 too low 280 cell 300 too diffuse?

* V525 one of those \neq voltage
so max the gate

going ~~error side~~ [→] ~~carburus~~ \neq distinguishing

V1 NDOOG% VD25 ~ 90% culture Cord ~ 13% culture
appended \rightarrow \uparrow events to record to 2.5 M

#3

Cord Fixed:

FSC/SSC is fine

\approx maybe down -10. \uparrow

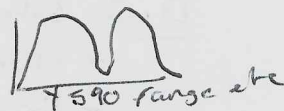
but adj. -sted the gate

Compensations are looking much much cleaner w/ current adjustments.
Voltage word def still needed ~~BE30~~ ^{BE30} 4590.

unfixed cord:

FSC: 580 \checkmark across board.

lots of spread out in the peaks



2% contrast @ 10:17pm

Done @ 11:25pm

September 9-10, 2021

HEU: P4b + P2 $\gamma\delta$ Cord Expansion D17

Hao-Ting & November	Ex vivo Gd-p4b	Surface μL	\times	Intra μL	\times
BV421	PD1	2	9		
BV510	Aqua L/D	1:500	-		
BV650					
Alexa 488 (FITC)	Perforin (dG9)	-	-	3	
PerCPeF710	CD3	1.5	6.3		
PE	GZMb	-	-	1.5	
PE Dazzle					
PE Vio770	NKG2A	0.6	2.7		
APC	CD56	1.3	5.85		
APC Fire750	VD2	1.2	5.4		
20.5 $\mu\text{L}/\text{rxn}$	PBS	11.9	62.6	17.0	

13.9

June	Ex vivo Gd-p2	Surface μL	\times	Intra μL	\times
BV421	VD2	1	4.5	2.5	
BV510	CD3	1.5	6.8	3.75	
BV650	NKG2D	2	5	5	
	Streptavidin	1.5	2.5	2.5	
Alexa 488 (FITC)	dg9	-	-	3	7.5
PerCPeF710	CD56	1.0	4.5	2.5	
PE	PD1	1.5	6.8	3.75	
PE Dazzle					
PE Vio770	NKG2A	0.6	2.7	1.5	
APC	VD1	1.0	4.5	2.3	
APC Fire750	Horizon L/D	1:1000	-		
20.5 $\mu\text{L}/\text{rxn}$	PBS	11.9	43.75	17.5	

$$\begin{array}{r}
 4 \\
 11.9 \\
 \times 2.5 \\
 \hline
 59.5 \\
 2380 \\
 \hline
 28.75
 \end{array}$$

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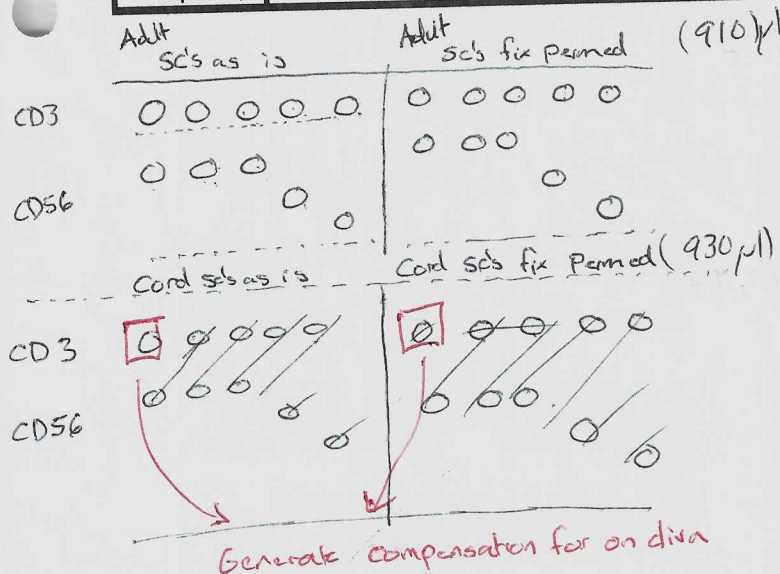
HEU: P4b + P2 $\gamma\delta$ Cord Expansion D17

Specimen	Status	<- ml	<- Conc	Tasks	Volume	Ly	2 1:100	Total	.5E+6	.3 E+6
170 μ l ND006	Adult				3.8	3.3E6		12.54	150 μ l	91 μ l
250 μ l CQ1070	D17 Cord				5.5	3.24E6		17.82	154 μ l	93 μ l

* Extra's get routed to Volitation experiments.

Current Version	Ex vivo Gd-p4b	Surface μ L	x 6.5 x 13	Intra μ L	x 20 x 30
BV421	PD1	2	13		
BV510					
BV650	CD56	1.8	11.7		
Alexa 488 (FITC)	Perforin (dG9)	-	-	3	60 μ l
PerCPeF710	CD3	1.5	9.8		
PE	GZMb	-	-	1.5	30
PE Dazzle					
PE Vio770	NKG2A	0.6	3.9		
APC	V82	1	6.5		
APC Fire750	Horizon L/D	1:1000	-		
20.5 μ l/rxn;	PBS	13.6	88.4	16	320 μ l

Test Aqua	Ex vivo Gd-p4b	Surface μ L	x 4.5 x 9	Intra μ L	x
BV421	PD1	2	9		
BV510	Aqua L/D	1:500			
BV650	CD56	1.8	8.1		
Alexa 488 (FITC)	Perforin (dG9)	-	-	3	
PerCPeF710	CD3	1.5	6.8		
PE	GZMb	-	-	1.5	
PE Dazzle					
PE Vio770	NKG2A	0.6	2.7		
APC	V82	1.0	4.5		
APC Fire750		13.6			
20.5 μ l/rxn;	PBS	13.6 61.2	16		



Test Aqua + CD16	Ex vivo Gd-p4b	Surface μ L	x 4.5 x 12.5	Intra μ L	x
BV421	PD1	2	9		
BV510	Aqua L/D	1:500			
BV650	CD56	1.8	8.1		
Alexa 488 (FITC)	Perforin (dG9)	-	-	3	
PerCPeF710	CD3	1.5	6.8		
PE	GZMb	-	-	1.5	
PE Dazzle					
PE Vio770	NKG2A	0.6	2.7		
APC	V82	1	4.5		
APC Fire750	CD16	1	4.5		
20.5 μ l/rxn;	PBS	12.6	56.7	16	