8/30/2021

Specimen	Status	<- ml	<- Conc	Tasks	Volume	Ly /2	#	Total	.5E+6	.3 E+6
ND006	2αL+ IL-2	0.8	4.06 E6 8.02 E6		1.3	4.99E6		6.49		
CQ1070 A	2αL+ IL-2	1.7	2.25 E6 4.5 E6		3.1	2.83E6		8.77	180 µl	110 µl -3 140 µl -4
CQ1070 B	2αL+ IL-2	1.8	2.12 E6 4.24 E6		3.3	2.82E6		9.30		

Neutrophils

Current Version	Ex vivo Gd-p4b	Surface µL	x 2	Intra µL	x 8
BV421	PD1	2			
BV510					
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	Horizon L/D	1:1000			
20.5 µl/rxn;	PBS	13.6		16	

Test Aqua	Ex vivo Gd-p4b	Surface µL	x 2	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	

Spin @ 1200 10 instead 1300 8

2:04 pm → starting $\frac{3}{4.6}$ x6 $\frac{2}{2}$ x1

14:29 L/D

2:31 sits in for 15 min # 7.96

14:48 (4 min late) → 1 PBS + 15% FBS for the 2ml wash after L/D. ¿effect on samples?

~ FBS coats L/D, so ½ conc que? ~ assuming titration to begin with...

3:03 pm → Surface stain → 3:23 pm sits done @ 3:12 pm ✓

Spin @ 3:26 pm

Fix Perm @ 3:37 pm

8($\frac{7}{1}$-3-1) x5 40min

Intracellular @ 16:17 → 16:57 pm

Done @ 5:14 pm

Test Aqua + CD16	Ex vivo Gd-p4b	Surface µL	x 2	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			16	

Main adj R780, BV510 + BV650 adj gain. + all high intensity set within my compensation gates

Hao-Ting & November	Ex vivo Gd-p4b	Surface μL	x 2	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	0.5			
APC	CD56	1.3			
APC Fire750	VD2	1.2			
20.5 μl/rxn;	PBS	12		17.0	

2'   1'   →630 for cells   start @ 5.30 pm   renamed to new naming
                                                       proper   convention.

540	630	FSC	620
300	300	SSC	300
520	555	R670	555
495	560	R780	560
585	540	B530	530
545	515	B710	515
480	460	V450	430
550	560 ←	V525 →460	
565	550	Y570 530	
660	641	Y540 630	
650	640	Y615 640	
525	515	Y780 510	

A: B530 smooshed neg pop.

6.30 min (some backflush into tube)

B)

B C)

B D E)   → UD2
           → gate missed ....

I am going to rerun the second set as "normal" file.
to see how playing with voltages /adj comp gate impacted
things....   Save V670 &670 adj CO56Δday.

I forgot to calculate
compensation, I edited
voltages, but didn't
calculate/apply until
after the fact.

[Figure out how to modify areas]

Don't diddle CST while
your are cytometer on...

← Like this
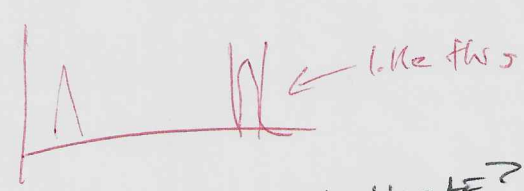
Some weird blips AF?
        when looking
        @ spillover controls

B530↓ B710 V458 Y590 Y280
        small ↓ adjustments.

B) Yep, apparentness of off charts visible again

GreenB — is the dead pop cells (way to dead?.....)