October 22-23rd, 2021

HEU: D17 γδ CBMC panels #4

	Specimen	Status	Date	Conc	Tasks	Volume <Div4>	Lymphocytes 1:50 (÷2)		Total	.66E+6	1E+6	.5E+6
430c/µl	Inf29-1	IL-2				6.8	5.4 E6	2.7E6	?	244µl	370µl	
480c/µl		201				6.9	4.7 "	2.3E6		287µl		217µl
570c/µl		BCG				6.8	5.6 "	2.8E6		236µl		179µl
650c/µl	Inf27-9	BCG				6.5	4.3 "	2.1 E6		314µl		238µl
		~~BCG~~										
900c/µl	Inf197-7	IL-2				6.8	1.4 "	0.7 E6		942µl	1.43ml	[illegible]
780c/µl		201				6.9	dead					
520c/µl		BCG				6.8	4.6 "	2.3E6		287µl		217µl
450c/µl	ND050	201				2.9	6.4	3.2E6				<.47 = 125µl

	Expanded Gd-p4b	Surface µL	x 6.5	Intra µL	x 6.5
BV421	PD1*	2	13		
BV510	Aqua L/D	1:500			
BV650	CD56*	1.8	11.7		
Alexa 488 (FITC)	Perforin (dG9)	-	-	3	19.5
PerCPeF710	CD3	1.5	9.8		
PE	-	-	-	-	
PE Dazzle					
PE Vio770	NKG2A	0.6	3.9		
APC	Vδ2	1	6.5		
APC Fire750	CD16	1	6.5		
20.5 µl/rxn;	PBS	12.6	81.9	17.5	113.8

	Expanded Gd-CK PMA	Surface µL	x 6.5	Intra µL	x 6.5
BV421	PD1	2	13		
BV510	Aqua L/D	1:500			
BV650	CD56	1.5	9.8		
FITC	CD107a	<3 µL added separately>			
PerCPeF710	TNFα			2	13
PE	Vδ2	1	6.5		
PE Dazzle					
PE Vio770	IFNγ	-	-	0.6	3.9
Alexa 647 (APC)	CD27	1.5	9.8	0	
APC Fire750	CD45RO	1.8	11.7		
20.5 µl/rxn;	PBS	12.7	82.6	17.9	116.3

← Fix these values

	Expanded Gd-p1	Surface µL	x 6.5
V450 BV421	PD1	2	13
V525 BV510	Aqua L/D	1:500	
V670 BV650	CD16	1.5	9.8
B530 FITC	Vδ2	1.2	7.8
B710 PerCPeF710	CD25	2	13
Y590 PE	CD28	2	13
Y615 PE Dazzle	CD27	1.5	9.8
Y780 PE Vio770	CD3	0.5	3.25
R670 APC	Vδ1	1	6.5
R780 APCFire750	CD45RA	1.8	11.7
20.5 µl/rxn;	PBS	7	45.5

	Expanded Gd-p2	Surface µL	x 6.5	Intra µL	x 6.5
BV421	Vδ2	1	6.5		
BV510	CD3	1.5	9.8		
BV650	NKG2D	2	13		
	Streptavidin	{1.5}	9.8	{19}	123.5
Alexa 488 (FITC)	dg9	-		3	19.5
PerCPeF710	CD56	1	6.5		
PE	PD1	1.5	9.8		
PE Dazzle					
PE Vio770	NKG2A	0.6	3.9		
APC	Vδ1	1	6.5		
APC Fire750	Horizon L/D	1:1000			
20.5 µl/rxn;	PBS	11.9	77.4	17.5	113.8

12:06 plate in
Ab started 1:40 pm
Abs done @ 2:20 pm
Plating @ 2:33-54
Cells spun @ 25C
L/D @ 3:12
Surface 15:47
Cells frozen @ 3:54 pm
16:02:35 → 4:17
Stop @ 4:?
4:38 pl/spin
CytFixPerm @ 4:55 pm
Intra @ 5:40
Fuck everything!

IL-2 197 ≠ pop wise
201 197 just dead

Exp #4 just not going well !!

18 → 36µl L/D Aqua
6-6µl L/D Horizon

cd ice @ 6:12
SAn @ 6:28 pm
L/D @ 6:43 → 6:58pm
TBS wash @ 7pm
Surface @ 7:11 pm (@ RT apparently !!)
FixPerm @ 7:45 pm (used up last of the bottle)
Intrac @ 8:26 pm
Done @ 9:15pm

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From #2, adjusted R780 B710 down 1g and shifted gate right

P2 640 (105 junk line NR 62D?)

~3 minutes.

↑ (really really obvious today (location CD3 VD2 smear?)

Something is wrong with our streptavidin.

(extra 20 minutes @ intracellular?)

(change in PermWash protocol?)

(last cytfix perm, ~~wash~~ bottle?)

(span down right before going forward?)

Well atleast it was Experiment #4

¿BC6 wells? ← appears to be these for 29/27 worst.

Not case for 197... strange...

No apparent ill effect to P4b

✡ P4b compensation bitoff for CD3 (B710) today ✡

P1 → 650 FSC. IC-2 added, for VD2 pop, long stretch.

10,000 evts/sec!! (200 evts/sec in)

Done ~2hr 5:17pm (2hr runtime) + turned off Canto