December 10-11th, 2021

Final round of 2021! And they all lived!

HEU: D17 γδ CBMC panels #12

Specimen	Status	Date	Conc	Tasks	Volume	Lymphocytes (1:50)	Lymphocytes ½	Total	.66E+6	1E+6	.5E+6
Inf212	IL-2										
	Zol					4.17	2.09		316 µl	478 µl	
	BCG					3.81	1.91		346 µl		262 µl
Inf187	IL-2					5.82	2.91		227 µl		172 µl
	Zol					3.37	1.69		390 µl	592 µl	
	BCG					3.98	1.99		332 µl		251 µl
N4062	Zol			Full 4:30 collection 8:10		4.86	2.43		272 µl		206 µl
						0.88	0.44				

4500c/µl
4500c/µl
4493c/µl
5600c/µl
5050c/µl
4300c/µl
1600c/µl

	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		
APC Fire750	CD45RO	1.8	11.7		
20.5 µl/rxn;	PBS	12.7	82.6	17.9	116.4

	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	3.3
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}	19.8	{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

Spin of .66 @ 10:51
Plate @ 11:35? 5:35pm
concerned 22 caps!!!
1.54pm spin
L/D @ 2:08 2:23
Surface @ 2:42 → 3:02
2:52 cells seen.
3:04 sets → 3:19
3:19 strept → 3:4

16 ml → 32 µl Aqua
6 ml - 6 µl L/D Horizon
54 ml Perm Wash
~ 80 ml FBS/PBS
- Last PFa/PBS

Actual L/D @ 6:15
higher background FBS?
mutal L/D stainers? Hal
Surface @ 6:40 → 7:00
Fix Perm @ 7:12
Intra @ 7:52 → 8:32
Done @ 8:43pm

spin @ 5:40pm
L/D @ 5:50 → 6:05
Was just FBS, the L/D is in the fridge!!!
celebrities tomorrow
200µl refills
caps?
paper towels
nonstripes
Stop... 15mn FBS wash & spin
3:38 sets pi down
Fix Perm @ 3:50
Intra @ 4:31
Done ~5:20
Lesigh...

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HEU: D17 γδ CBMC panels #12

Last saturday death marsh, SC's are all HEU-to zol since NYO62 failed expand "LSR" recently fixed as far as sheathe capacity pressure... supposedly.

FSC 520 high V525 out from voltage set.

Y590 PE → Y615 $10^{4.2}$ ✓

V670 weird stretch, overlap on VV52 sig.?

V750 collapsed muted peak?

¿What information about brightness/adequacy hidden in my SCs?

¿What about biological?

¿what about technical? [PMT]

Running p46 on FSC 610, pd1 + CD56 margins ✓

Vortex speed ↓6.5 (foaming tube)

What big picture stuff do we loose in our technique routine/setup

☆ p46 Inf187 IL-2 can't be right, also running on 5 min stims ☆

zol ✓ (just higher baseline?) just easily passed 17,000 @ 1 minute mark.

Inf187 3K 10K

BC6 + IL-2 switched???

Am glad insisted on IL-2 baseline ✓

VD2 count:

Inf 212	0.6	9.5	1.3 [% L/D]
Inf 187	~~3.3~~ 2.1%	27.2%	~~2.5%~~ 1.8

based on frequency should be able to locate other corresponding sets.

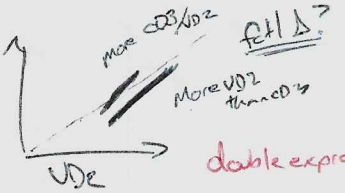
What they pop changes to confirm.

☆ The cell shift @ acquire raises, coincides w/ stabilizing Threshold rate ☆

P1 IL-2 Inf 212 FSC @610 to see?

% L/D culture might alter w/ surface vs FixPerm panels.

≠ case w/ Inf187... CD3



fd1 Δ?

double expressors?

☆ 187 IL-2 P1 also running close to 5 minutes ☆

¿de cells were IL-17 poised? .... check Mosteanu paper

"reality is I consider most you what hypocrites"