

October 10-12th, 2021

HEU: D17 $\gamma\delta$ CBMC panels - Test A

Specimen	Status	Location	Conc	Date	Tasks	Volume	Lymphocytes	EL6	460	5
INF018	IL-2				1 vial 15M frozen concy	6.1	2.5E6	15.25	264 μ l	200 μ l
	201				1 vial 15M frozen concy	6.4	2.49E6	15.90	265 μ l	200 μ l
	BC6				1 vial 15M frozen concy	6.3	2.65E6	16.69	249 μ l	188 μ l (219 concy)
NY062	201				Agate for concy	2.5	1.82E6	4.55		219 μ l

2700 μ l
3000 μ l
3600 μ l
~1300 μ l (agitate)

~10M frozen concy
H=1691
Just enough cells

	Expanded Gd-p4b	Surface μ L x 3	Intra μ L x 3
BV421	PD1*	2 6	
BV510	Aqua L/D	1:500	
BV650	CD56*	1.8 5.4	
Alexa 488 (FITC)	Perforin (dG9)	- -	3 9
PerCPeF710	CD3	1.5 4.5	
PE	{GZMb}	- -	1.5
PE Dazzle			
PE Vio770	NKG2A	0.6 1.8	
APC	V82	1 3	
APC Fire750	CD16	1 3	
20.5 μ l/rxn;	PBS	12.6 37.8	17.5 52.5

	Expanded Gd-CK PMA	Surface μ L x 3	Intra μ L x 3
BV421	PD1	2 6	
BV510	Aqua L/D	1:500	
BV650	CD56	1.5 4.5	
FITC	CD107a	<3 μ L added separately>	
PerCPeF710	TNF α		2 6
PE	V82	1 3	
PE Dazzle			
PE Vio770	IFN γ	- -	0.6 1.8
Alexa 647 (APC)	CD27	1.5 4.5	0
APC Fire750	CD45RO	1.8 5.4	
20.5 μ l/rxn;	PBS	12.7 38.1	17.9 53.7

	Expanded Gd-p1	Surface μ L x 3
V450 BV421	PD1	2 6
V525 BV510	Aqua L/D	1:500
V670 BV650	CD16	1.5 4.5
B530 FITC	V82	1.2 3.6
B710	CD25	2 6
PerCPeF710	CD28	2 6
Y590 PE	CD27	1.5 4.5
Y615 PE Dazzle	CD3	0.5 1.5
Y780 PE Vio770	V81	1 3
R670 APC	CD45RA	1.8 5.4
R780 APC Fire750	PBS	7 21

	Expanded Gd-p2	Surface μ L x 3	Intra μ L x 3
BV421	VD2	1 3	
BV510	CD3	1.5 4.5	
BV650	NKG2D	2 6	
	Streptavidin	{1.5}	
Alexa 488 (FITC)	dg9	-	3 9
PerCPeF710	CD56	1 3	
PE	PD1	1.5 4.5	
PE Dazzle			
PE Vio770	NKG2A	0.6 1.8	
APC	VD1	1 3	
APC Fire750	Horizon L/D	1:1000	
20.5 μ l/rxn;	PBS	11.9 35.7	17.5 52.5

11:12 pm cells in - TCR plate
1:03 pm \rightarrow cells spin down
1:22 pm L/D
1:32 pm scb
surface @ 1:56 pm
56/FD1 5.0 @ 2:07 pm
step @ 2:46 pm
14:58 cells frozen back
15:13 cells fix/pern
Intra @ 3:57 pm
P2/P4/P6 @ 4:53 pm

400 μ l
C8
7.2 \rightarrow 7.8 \rightarrow 7.16 μ l
3-3
20-3-27 permeabil
40 ml FBS-PBS

5:26 spin down
40 @ 5:36
surface @ 6:01 pm
Fix Perm @ 6:41 pm
Intra @ 7:23 pm
Donor @ 8:14 pm

Genix FBS 100-106
Lot A174747
For freezing expansion orange label
green tape
lower right notch
 \rightarrow boxes back

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Hao-Ting ran on LsR once pump/blue laser were fixed.

Issue of 40% viability $\frac{1}{2}$?

Also IL-2 needs more than 500K
as $gd \approx 0.2\%$ $IEC \times 50\% \times 0.2\%$

800K - IEC for IL-2 wells
500K for 2d/BCG.

What about for TCR stim condition?

Plate prep
1:100 PBS 50 μ l well

$$\begin{array}{r} 150 \\ \times 3 \\ \hline 450 + 450 + 450 + 150 \\ \hline 1700 \mu\text{l} \\ \hline 17 \mu\text{l} \end{array}$$

1600 ml in 16 μ l
1584 μ l + 16 μ l

Spinning down $\sim 12.5M$ /tube

resuspending in 250 ml FBS - ~~DBS~~
 ~ 750 ml

+ 250 ml 20% DMSO-FBS

1 ml $\sim 800 \mu$ l + 200 μ l

6/21/21 Penn Wash empty

\hookrightarrow 10/11/21 ~ 4 months

Almost forgot the 2 sc's for PD1/56 adjusting

120 100 120

2M cells/ml

660K/330 μ l

330-residual

R10 + golgi

+ 3 μ l CD107a

2 min 1000 rpm

2 ml RPMI + 2 μ l Bref + 1.3 μ l Golgi stop medium

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