

November 8th, 2021

Perm wash ✓
DI #20?
... y or n

HEU: Ex-vivo γδ CBMC panels #9

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	2E+6	1.5E+6
Inf149-4 A-2 (11)	SW1 HU	Box SA, GS		2019 01-16		3	3.1 E6	4.07 E6	10.83	Combine 2?	484 μl
Inf 282-2 a-2 (7)	SLP6 HEU-L	Box SA, F.5		2019- 08-02		3	3.42 E6	6.22 E6	10.26	509 μl 373 μl	439 μl
Inf180-9 a-2 (11)	SAN7 HEU-hi	Box GA H1		2019- 02-07		2	5.05 E6	6.60 E6	10.1	509 μl 373 μl 252 μl	297 μl
NY062	Adult		20E+6	03-12- 2021		3	4.53 E6	6.36 E6	13.59		

	Ex vivo Gd-p4b	Surface μL x 3	Intra μL x 3
BV421	PD1	2	6
BV510	Aqua L/D	1:500	
BV650	CD56	1.8	5.4
Alexa 488 (FITC)	Perforin (dG9)	-	3
PerCPeF710	CD3	1.5	4.5
PE	GZMb	-	1.5
PE Dazzle			4.5
PE Vio770	NKG2A	0.6	1.8
APC	V82	1	3
APC Fire750	CD16	1	3
20.5 μl/rxn;	PBS	12.6	37.8

	Ex vivo Gd-CK PMA	Surface μL x 6.5	Intra μL x 6.5
BV421	PD1	2	13
BV510	Aqua L/D	1:500	
BV650	CD27	1.5	9.75
FITC	V82	1.2	7.8
PerCPeF710	CD3	1	6.5
PE	CD56	0.5	3.25
PE Dazzle			
PE Vio770	IFNγ	-	0.6
Alexa 647 (APC)	TNFα	-	2
APC Fire750	CD45RO	2	13
20.5 μl/rxn;	PBS	12.3	80

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

	Ex vivo Gd-p2	Surface μL x 3	Intra μL x 3
BV421	V82	1	3
BV510	CD3	1.5	4.5
BV650	NKG2D	2	6
Alexa 488 (FITC)	Streptavidin	{1.5}	{19}
PerCPVio700	dg9	-	3
PE	CD56	1	3
PE Dazzle	PD1	1.5	4.5
PE Vio770			
APC	NKG2A	0.6	1.8
APC Fire750	V81	1	3
20.5 μl/rxn;	Horizon L/D	1:1000	

Perm wash: 22 x 3 = 66 (concentration) 50 + 30

FBS 48 + 70 ~ atleast 2

25 x 3 = 75 10 ml RBC lye 1-2

10 L/D entire NER 3-3

4 μl → 10 μl

12.8 10-20

4/D @ 8:47 → 9:02

first RBC wash ✓

PMA surface @ 9:35 → 9:55 pm

100 μl @ 9:50

Fix Perm @ 22:37 pm

wash 1 ✓ wash 2 ✓

Intra @ 23:20

(Done @ 1:25 am)

- 12:29 - Cell Count

12:23 pm - PMA in

intra @ 2:53 pm

3:23 up to 3:35 pm

concentrations!

At 5:41 pm

4:00 @ 6:33 pm

Spn @ 6:55

7:09 tetra

for base

Surface @ 7:14

RBC lye @ 7:30 pm

SD @ 7:46 →

8:01

Step @ 7:54 pm

8:01

Set's the lye @ 8:10

8:10

PMI spn @ 8:50 pm

Fix Perm @ 8:40 →

9:00

first perm wash ✓

Per/Perm intra @ 9:40

Per/Perm 10:00

November 8-9th, 2021

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Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	1E+6	1.5E+6
Inf 149-4 a-4 (11)	SWER HU					1.5	5.53	7.12 E6	<u>8.3</u> 10.7		+580 420/3E+6
Inf 282-2 a-2 (7)	SLP7 HEU-10					2	5.36	8.99 E6	10.72 17.99		+670 330/3E+6
Inf 180-1 a-1 (11)	SRNG HEU-hi					2	7.45	9.70 E6	<u>15.9</u> 19.40		+700 300/3E+6
								6.3 E+6			2400/3E+6

PMA's

$$484 + \underline{216}$$

$$439 + \underline{261}$$

$$297 + \underline{403}$$

$$\begin{array}{c} a_2 \\ \downarrow \\ a_1 \end{array} \quad \begin{array}{c} \text{leftover} \\ + \end{array} \quad \begin{array}{c} a_2 \\ \downarrow \\ a_1 \end{array} \quad \begin{array}{c} 484 \\ + \end{array} \quad \begin{array}{c} 216 \end{array}$$

$$a_2 \quad 373 \quad + \quad 327$$

$$a_2 \quad 252 \quad + \quad 448$$

$$6 \times 300 = 1800 (1900?)$$

$$(5.5) \quad 200\mu l = 1.1$$

$$200\mu l = 0.75$$

$$C_1 V_1 = C_2 V_2$$

$$\frac{1.1}{1.5} = (1.5) \times \underline{730} \quad 1.5\mu l$$

$$\frac{.75}{1.1} = \underline{500} = 1\mu l$$

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