

November 23-24th, 2021

HEU: Ex-vivo $\gamma\delta$ CBMC panels #12

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly E6	Ly+Mon	Total	2E+6	1.5E+6
Inf 030-3 a-2 (a)	RRBL HU	Box 1A, F.9		2018- 08-01	clump	2	1.75	2.17 E6	3.5	—	857 μ l
Inf 212-5 a-2 (c)	SB6B Heu-h	Box 2A, E.5 "3"		2019- 07-27		5	3.24	4.99 E6	16.2	617 μ l	463 μ l
Inf 187-6 a-2 (7)	SA1T Heu-h	2018 Box 6A, F.1		2019 02-05		4	3.39	5.27 E6	13.6	590 μ l	442 μ l
N4062	Adult		20 E6	2021- 03-18		3	4.30	6.42 E6	12.9		<47 = 93 μ l

	Ex vivo 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
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	16.0

	Ex vivo Gd-CK PMA	Surface μ L x 6.5	Intra μ L x 6.5
BV421	PD1	2	13
BV510	Aqua L/D	1:500	
BV650	CD27	1.5	9.8
FITC	V82	1.2	7.8
PerCPeF710	CD3	1	6.5
PE	CD56	0.5	3.3
PE Dazzle			
PE Vio770	IFN γ	-	0.6
Alexa 647 (APC)	TNF α	-	2
APC Fire750	CD45RO	2	13
20.5 μ l/rxn;	PBS	12.3	17.5

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

	Ex vivo Gd-p2	Surface μ L x 3	Intra μ L x 3
BV421	V82	1	3
BV510	CD3	1.5	4.5
BV650	NKG2D	2	6
	Streptavidin	{1.5}	{19}
Alexa 488 (FITC)	dg9	-	3
PerCPVio700	CD56	1	3
PE	PD1	1.5	4.5
PE Dazzle			
PE Vio770	NKG2A	0.6	1.8
APC	V81	1	3
APC Fire750	Horizon L/D	1:1000	
20.5 μ l/rxn;	PBS	11.9	17.5

4x8 = 3.2 ml w/o NIR

12.8 = 9.6 ml w/o Aqua (20)

3 ml - 3 μ l w/o Heu

PNA: 4:08:03 pm

Surface: 8:40 pm

Ficoll: 9:25 (+ unstained alk controls)

35 → 45

2nd perm wash

Intra: 22:09

Wash wash 22:49

stain@11:23 am

PNA@1:42 pm

Abs prep@5:10 pm

6:28 pm spindel

6:41 mid prep

6:51 tris buffer

6:58 7.13

7:05 7:20

7:29 pm Surface

→ 49

strep@8:12

Ficoll@8:42

Intra@9:30

done@10:49 pm



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Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	1E+6	1.5E+6
Info30-3 a-2 (9)	PRBN HU			2018-08-01	clump1	2	1.8	2.22 E6	3.6 4.4	—	833 μ l
Inf 212-5 a-1 (6)	SB6A HEU-L			2019-07-27		5	3.8	5.55 E6	19 27.8	850 μ l + 150 3E6	394 μ l
Inf 127-6 a-1 (7)	SALS HEU-H			2019-02-03		4	4.44	6.54 E6	18 26.2	460 μ l + 540 3E6	334 μ l
								6.42			
											240 μ l + 760 1.5E+6

Yellow-B Fluorescence (YEI-B-HLog) PE 583/26

< 212 μ l 200 μ l for ordered + 200 μ l R10
+ 110 μ l max/ \rightarrow 650 μ l cells

$$CD127 \times .8 = 3.2$$

$$CD161 \times .8 = 3.2$$

$$CD107A \times 6 = 24$$

$$\frac{20.5}{7.2}$$

$$\frac{13.3 \times}{4}$$

$$\boxed{53.2} \text{ R10}$$

$$\frac{857 + 143}{\swarrow 2}$$

$$463 + 237$$

$$442 + 258$$

$$\frac{100 = .3 \rightarrow .6}{300 = 1}$$

* BCG 5 vial = $1.3 \text{ E}6 \text{ CFU/ml} \times 5$
[2 well conditions] = $6.5 \text{ E}6 \text{ CFU}$

after wash
resuspend in 2.16 ml R10 ($\sim 3 \text{ E}6/\text{ml}$)

1.5 M

2 μ l/ml

Final
0.650 \rightarrow 1.3 M/ml

NIT -

$$617 + 83$$

$$590 + 110$$

$$\frac{4}{\times 3} = 1200$$

Major FSC change \rightarrow 520, everything else exactly same as #11
 \rightarrow new sip tube plays off at tube placement.

Ideally, nowhere else for V450 to go! its mixed out.

giving 5 secs pre washing

Y615... come on! (absorbed sample resuspended enough in the last droplet....)

13.52	13.78
.07	.01
.27 \rightarrow	.35
.00	.00
.05	.03
<u>11.52</u>	<u>11.95</u>

I90 Fsc for Inf030 P2 (accidentally started on p4b switch)
 \uparrow and forgot to record... AmI.
 thankfully unexposed....

212 \rightarrow 2:49 sec

CD45- reticulocytes issue

P4b may be \rightarrow than P2

As far as how far down needle/sip goes LSR/Aurora

stripe in Inf30 P4b/CD56.

* About 5 sec for FSC to catchup to equilibrium *

(Bubbles & ghost cells)



* Info 30 not a bit of Vd2's * Added 2 pbs drops to 212 ctrl to extend runtime

Nothing on PMA 212.... what the hell.

* monitor for cracks going forward

transferred to another tube. Let it ride!

It's backfilling... 212 PMA.

\uparrow 2 cracks in the tube



\uparrow speed to high. 1500 c/s/sec

vs a normal sample.

brought p1 down to 570 (off charts)

\downarrow missing CD 25? nope just that 30 specimen

