

September 24-25th, 2021

HEU: Ex-vivo $\gamma\delta$ CBMC panels Test A

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	1E+6	1.5E+6
Inf 18-9 RLT 1a-2	HU	Box 1A S4, CS			staining RNA	4	3.13 E6	4.26 E6	12.52 17.04	349 μ l	479 μ l
Inf 18-9 RLT 6a-2	HU	Box 1A C4			culture	3	2.3 EC	3.3 EF	6.9 9.9		
NY062	Adult		20E 6	08/12/21	Single color controls RNA	3	4.53 E6	6.34 E6	13.54 19.02	BOOR = 67 μ l	
+2 BEGwi tubes											

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

	Ex vivo Gd-CK PMA	Surface μ L x 2	Intra μ L x 2
BV421	PD1	2	4
BV510	Aqua L/D	1:500	
BV650	CD27	1.5	3
FITC	V82	1.2	2.4
PerCPeF710	CD3	1	2
PE	CD56	0.5	1
PE Dazzle			
PE Vio770	IFN γ	-	-
Alexa 647 (APC)	TNF α	-	-
APC Fire750	CD45RO	2	4
20.5 μ l/rxn;	PBS	12.3	24.6

	Ex vivo Gd-p1	Surface μ L x 1
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		7
APC Fire750		7
20.5 μ l/rxn;		

	Ex vivo Gd-p2	Surface μ L x	Intra μ L x 1
BV421	VD2	1	1
BV510	CD3	1.5	1.5
BV650	NKG2D	2	2
	Streptavidin	{1.5}	
Alexa 488 (FITC)	dg9	-	-
PerCPVio700	CD56	1	1
PE	PD1	1.5	1.5
PE Dazzle			
PE Vio770	NKG2A	0.6	0.6
APC	VD1	1	1
APC Fire750	Horizon L/D	1:1000	
20.5 μ l/rxn;	PBS	11.9	11.9

At the prep @ 7:17
Abs ✓ 8:06 am
Thaw @ 10:34
Stain @ 10:53
L/D @ 12:04 pm
→ 12:31 pm
= 22 min
1:07 RBC
lysis in ✓
spin w/ 1.5 ml
5% FBS- PBS
@ 13:11
SCS, @ 1:20 →
#1.55

CKPMA in @ 11:42 am → 5:42 pm
2 L/D horizon 1.6 → 3.2 aq
L/D @ 5:55 pm
Surface @ 6:00 pm
Abelise @ 6:41 pm
C/Fix Rem 6:54 pm
@ 19:35 Intra cell

Strop 5 in @ 1:33 holding p46
→ 1:48 for nore 48
SCS spin @ 1:38 pm
2:00 trizol
2:03 CytRifPeri
Tried into -80°C @ 2:21
Intra @ 2:45 → 3:34 pm
Done @ 3:40 pm

1-8-10
5-10
10-1

10-1

10-1
10-1

500 μ l + 500 μ l
+ R10 R10 + cells
(+2 μ l) @ 3M

10-1
10-1
10-1

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BGG \rightarrow 120 ~ 2 ml / donor
~ 6 ml / 3 donor

Water bath, swirl

Span 4000 g 5 button, switch G, adjust up \rightarrow 5 minutes

2 ml
 2 ml
 20 ml
 1 ml (↑ 2 ml / ml add BCC)
 1 ml add
 $\text{①} + 10 \text{ ml}$
 $(20 \text{ ml}) \times \frac{100}{2 \text{ ml}} = 20 \text{ mg/ml}$
 $(6.7 \text{ ml}) \times \frac{1.30}{1 \text{ ml}} = 13.3 \text{ mg/ml}$
 $\frac{20 \text{ mg/ml}}{1.30} = 15.38 \text{ mg/ml}$
 $\frac{15.38 \text{ mg/ml}}{1 \text{ ml}} = 15.38 \text{ mg/ml}$
 1 Buge needle
 3 ml storage
 5 needles

