

June 11-12th, 2021

HEU: New $\gamma\delta$ Cord Panels Experiment #1

Ex-vivo panel optimization & expansion for RT-PCR

Ran 6/11/2021 7-8 pm

1:00 dilution 15

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	1.5E+6	2E+6
Inf250-7 SFM 2-1	HIV (H) Unspliced	9AD2									
Inf250-7 SFM3 2-2	HU	9AD3				6 ml		2.67E4	4.134		
Inf250-7 SFM 4		9AD4				5 ml		3.5E4	5.32E4		
NY 3			5E7	6-17-14		1 ml		1.31E5	1.09E5		
Inf250-7 SFM5		9AD5									

✓	Ex vivo Gd-p4b	Surface μL x 1	Intra μL x 1
BV421	PD1	2.0	
BV510			
BV650	CD56	1.8	
Alexa 488 (FITC)	Perforin (dg9)	-	3
PerCPeF710	CD3	1.5	
PE	GZMb	-	1.5
PE Dazzle			
PE Vio770	NKG2A	0.6	
APC	V82	1.0	
APC Fire750	Horizon L/D	1:1000	
20.5 $\mu\text{L}/\text{rxn}$;	PBS	13.6	16

✓	Ex vivo Gd-CK PMA	Surface μL x 2.5	Intra μL x 2.5
BV421	PD1	2	5
BV510	Aqua L/D	1:500	
BV650	CD27	1.5	3.75
FITC	V82	1.2	3.0
PerCPeF710	CD3	1	2.5
PE	CD56	0.5	1.25
PE Dazzle			
PE Vio770	IFN γ	-	0.6
Alexa 647 (APC)	TNF α	-	2
APC Fire750	CD45RO	2.0	5.0
20.5 $\mu\text{L}/\text{rxn}$;	PBS	12.3	30.75

✓	Ex vivo Gd-p1	Surface μL x 1
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 APCFire750	CD45RA	1.8
20.5 $\mu\text{L}/\text{rxn}$;		7

✓	Ex vivo Gd-p2	Surface μL x 1	Intra μL x 1
BV421	V82	1	
BV510	Aqua L/D	1:500	
BV650	CD56	1.8	
Alexa 488 (FITC)	Perforin (dg9)	-	3
PerCPeF710	CD3	1.5	
PE	NKG2D	0.6	
PE/Dazzle			
PE Vio770	NKG2A	0.6	
APC	V81	1	
APC Fire750			
20.5 $\mu\text{L}/\text{rxn}$;		14.0	17.5

16:00 minute on time

540 FSC adjustment.

MAN
@ 9:36 PM
3:36 PM
11:12 →
11:27 PM
Sdsce 270
→ 420
Surface
1:45 →
12:05 PM
12:24
Cytex
FAM
P1 Data

JK L/D @
3:47
Surface @ 4:15
Intro @
5:28 PM
→ 6:08 PM
Cleaning Labssa
@ 6:44 PM

$$\left[\begin{array}{l} 2 \mu\text{l PMA-Ionomycin} \\ + \text{gels stop} \end{array} + (500 \mu\text{l}) R_{10} \right] + \left[\begin{array}{l} \square \\ \times \# \text{ specimens (PMA)} \end{array} \text{ Million cells} + 500 \mu\text{l } R_{10} \right]$$

(ditto for 2 μl of Gels stop control) = $1 \mu\text{l PMA/Ionomycin/ml}$ 625 μl

From our calculated concentration \leadsto 1.5 million cells \leadsto 3.0 million cells/ml
 Plated total 500 μl then plate 500 μl

> conc than 3EC/ml, plate 232 μl
 See ex 4 $\frac{1.5}{4} = \text{volume pl + whatever } \uparrow 500 \mu\text{l}$

$\sim 70 \mu\text{l DNase / cord.}$

2.4 million cells/ml

375 μl

625 μl

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350 add'l
1.31 ET ~ 4.5M
350 2.67 ~ 8.00K
1.5 3.5 4.5.25