

April 22th, 2021

HEU: $\alpha\beta$ Panel Optimization + NKT panels

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+M	Total	1.5E+6	2E+6

Cytokines Panel B	Ex vivo $\alpha\beta$ T cells	Surface μL x		Intra μL x	
V450 BV421	CD4	1.8		0.5	
V525 BV510	Aqua L/D	1:500			
V670 BV650	CD8	1.5		0.5	
B530 FITC	CD45RA	1.5			
B710 PerCPeF710	TNF α	-		3.0	
Y590 PE	CD62L	2			
Y615 PE Dazzle	-	-			
Y780 PE Vio770	IFN γ	-		0.8	
R670 APC	CD3	1.5		0.5	
R780 APC Fire750	CD69	1.0		1	
20.5 $\mu\text{L}/\text{rxn}$;	PBS	11.2		14.2	

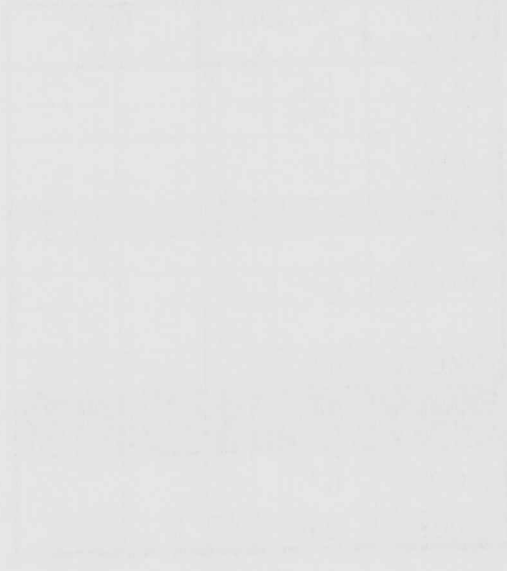
Cytokines Panel C	Ex vivo $\alpha\beta$ T cells	Surface μL x		Intra μL x	
V450 BV421	CD4	1.8		0.5	
V525 BV510	Aqua L/D	1:500		-	
V670 BV650	CD45RA	1.5		-	
B530 FITC	CD69	1.0		1.0	
B710 PerCPeF710	TNF α	-		3.0	
Y590 PE	CD62L	2			
Y615 PE Dazzle					
Y780 PE Vio770	IFN γ	-		0.8	
R670 APC	CD3	1.5		0.5	
R780 APC Fire750	CD8	1.5		0.5	
20.5 $\mu\text{L}/\text{rxn}$;	PBS	11.2		14.2	

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Intracellular Test	Failed NKT	Surface μL x	Intra μL x
BV421	CD4	1.8	
BV510	L/D	1:500	
BV650	CD56	1.8	
FITC	dg9		3
PerCPeF710	CD3	1	
PE	NKG2D	0.6	
PE Dazzle			
PE Vio770	NKG2A	0.6	-
Alexa 647 (APC)	hCD1d	0.6	-
APC Fire750	CD8	1.5	
20.5 $\mu\text{L}/\text{rxn}$;	PBS	12.6	

Surface Test	Failed NKT/MAIT	Surface μL x
V450 BV421	Va7.2	2
V525 BV510	Aqua L/D	1:500
V670 BV650	Strep	1
	CD26	1
B530 FITC	CD4	1.8
B710 PerCPeF710	CD45RA	2
Y590 PE	CD161	0.5
Y615 PE Dazzle	CCR7	1.5
Y780 PE Vio770	CD3	0.5
R670 APC	CCR6	2.5
R780 APCFire750	CD8	1.5
20.5 $\mu\text{L}/\text{rxn}$;	PBS	8.7



February 11-13th, 2021

Degranulation/NKT tetramer tests

Degranulation TNF α	Ex vivo A β T cells	Surface μ L x		Intra μ L x	
V450 BV421	CD69	0.5		1	
V525 BV510	Aqua L/D	1:500			
V670 BV650	CD4	1.8		0.5	
B530 FITC	CD107A	<3ul added separately>			
B710 PerCPeF710	TNF α	-	-	3	
Y590 PE	CD62L	2			
Y615 PE Dazzle					
Y780 PE Vio770	CD8	1.5		0.5	
R670 APC	CD3	1.5		0.5	
R780 APC Fire750	CD45RA	1.5			
20.5 μ L/rxn;	PBS	11.3		15	

Degranulation IFN γ	Ex vivo A β T cells	Surface μ L x		Intra μ L x	
V450 BV421	CD69	0.5		1	
V525 BV510	Aqua L/D	1:500			
V670 BV650	CD4	1.8		0.5	
B530 FITC	CD107A	<3 ul added separately>			
B710 PerCPeF710	CD3	1.5		0.5	
Y590 PE	CD62L	2			
Y615 PE Dazzle					
Y780 PE Vio770	IFN γ	-	-	0.8	
R670 APC	CD8	1.5		0.5	
R780 APC Fire750	CD45RA	1.5			
20.5 μ L/rxn;	PBS	11.7		17.2	

Intracellular Test	NKT	Surface μ L x		Intra μ L x	
BV421	CD4	1.8		0.5	
BV510	L/D	1:500			
BV650	CD56	1.8			
FITC	dg9			3	
PerCPeF710	CD3	1		0.2	
PE	NKG2D	0.6			
PE Dazzle					
PE Vio770	NKG2A	0.6	-		
Alexa 647 (APC)	hCD1d	0.6	-		
APC Fire750	CD8	1.5		0.5	
20.5 μ L/rxn;	PBS	12.6		16.3	



April 22 2021

7:02 Out
7:23 Abs found
8:00 Abs prepared
8:16 L/D

Panel B

		Extra	(x 3.5)	Intra	(x 3.5)
BV421	CD4	1.8	6.3	0.5	1.75
BV510	L/D 2 μ /ml ~ 11500	-	-	-	-
BV650	CD8	1.5	5.25	0.5	1.75
Fic	CD45RA	1.5	5.25	-	-
PerCP	TNF	-	-	3.0	10.5
→ PE	CD62L	2	7.0	-	-
Pe-Vio770	IFN γ	-	-	0.8	2.8
APC	CD3	1.5	5.25	0.5	1.75
APC/Fic	CD69	1.0	3.5	1.0	3.5
PBS		11.2	39.2	(14.2)	49.7

Panel C

		(x 3.5)	x 3.5
OUT4 Bldg BV421	CD4	1.8	6.3
BV510	L/D	-	-
H1400 Bldg BV650	CD45RA	1.5	5.25
FN50 Fic	CD69	1.0	3.5
Ma611 Bldg PerCP	TNF	-	-
DRE650 Bldg PE	CD62L	2	7.0
45-15 Milkst Bldg Pe-Vio770	IFN γ	-	-
OK-B Bldg APC	CD3	1.5	5.25
RPA-T8 APC/Fic	CD8	1.5	5.25
PBS		11.2	39.2

1.6 μ l IFN γ
2.0 μ l IL-4/tube

1:100
1:200

2300/11
2.47
x 2.5
1235
4840
6175

OH NRT Sal

16.175

3.082

BV421	CD4	1.8
BV510	40	1:500
BV650	CD56	1.8
Ftc	CD3 dg9	-
PerCP	NK620 CD3	1.0
YS90	NK62A NK620	0.6
Y180	CD1d NK62A	0.6
APC	CD1d	0.6 (1:200)
APC Fc	CD8	1.5
BB5		

2ml 5% FBS
30min 1400 G
CS Interact

add sc

12.6

8.9

Modified Mart.

BV421	Va. 7.2	2
BV510	40	1:500
BV650	CD26	1ul brain / 1ul Saeep
Ftc	CD4	0.8
PerCPeF716	CD45RA	2.0
PE	CD161	0.5
Pe/Dazdc	CCR7	1.5
PeVio 780	CD3	0.5
APC	CD1d (cccr)	??
APC eF780	CD8	1.5

3 wells

if CD8 = 2.5

8.7

Start @ 2:19pm

2:33pm + 15 = 2:48

2:41 + 15 = 2:56

interact wash @

strep @ 4:10? Penicillin @ 4:20
PFA for WIT @ 4:20