

August 4-5, 2021

HEU: Innate-like T cell Panels  
Ex-vivo MAIT & NKT tetramer dilutions

# Tetramer Aliquots:

Large brown bag spilled, need to autoclave. used 3 of those for hCD1d APC, sid Fite, sid APC At 3

MRI FP Fite ~ 130  $\mu$ l - aliquoted 30  $\mu$ l

All @ 30  $\mu$ l aliquots past this point, For MRI-ORRU Fite/APC, moved 10  $\mu$ l from At 3 to stock for today's.

Fite MRI ORRU 1.5 mg/ml

APC MRI ORRU 1.5 mg/ml

Fite MRI-FP 1.5 mg/ml

APC MRI-FP 1.5 mg/ml

Fite hCD1d 57 PBS 1.6 mg/ml

APC hCD1d PBS 57 1.6 mg/ml

Fite hCD1d solo 1.6 mg/ml

APC hCD1d solo 1.6 mg/ml

1.5

h6	1.5 $\mu$ g	1 $\mu$ l	100 $\mu$ l	1:100
0.8 $\mu$ g	0.75 $\mu$ g	0.5 $\mu$ l		1:200
0.32 $\mu$ g	0.3 $\mu$ g	0.2 $\mu$ l		1:500
0.16 $\mu$ g	0.15 $\mu$ g	0.1 $\mu$ l		1:1000
	0.075 $\mu$ g	0.05 $\mu$ l		1:2000

08/05/2021 / Face-Mask

being remanded starting tomorrow.

"doesn't exist in LSR-II (David-Rach)  
David, 3y Rach

## Mant

2 Fite	2 APC		
or		(x 2)	
2(1+1) conc	+ 2(1+1) sep	NCT	
3	4	3	4
x 8	x 8	x 4	x 4
24	32	12	16
x 1.5	x 1.5	x 1.5	x 1.5
36M	48M	18M	24M

8 + 8

12 + 12

Post tetramet, map

APC cd1d	APC hCD1d	APC solo	
1:200	1:1000	1:200	
FP 1:200	FP 1:500	MAITFP 1:1000	APC solo 1:500
ORRU 1:200	ORRU 1:500	APC MAITRU 1:1000	APC solo 1:1000

# Compensation Log:

Titrate down Fite OD8?

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Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+M	Total	1.0E+6	2E+6
ND050A			15E6	03/31/21		3	3.17E6	5.2E6	9.51	315f	95f
ND050B			15E6	03/31/21		3	3.16E6	5.2E6	9.48	315f	95f
ND050C			15E6	03/31/21		3	3.47E6	5.5E6	10.4	290f	86f

Now out of ND050 PBMCs !!

Reference - Actual MAITs

	Ex vivo MAIT-p1a	Surface μL x
V450 BV421	Va7.2	2
V525 BV510	Aqua L/D	1:500
V670 BV650	Strep CD26	1
B530 FITC	CD4	1.5
B710	CD45RA	2
PerCPeF710	CD161	0.5
Y590 PE		
Y615 PE Dazzle		
Y780 PE	CD3	0.5
Vio770		
R670 APC		
R780	CD8	1.5
APCFire750	PBS	12.7
20.5 μl/rxn;		

Tetramer Test A1	Ex vivo MAIT-tet	Surface μL x
V450 BV421	CD4	1.8 23.4
V525 BV510	Aqua L/D	1:500 -
V670 BV650		-
B530 Alexa488 (FITC)	(3) Tetramer (1,2,5)	
B710	CD45RA	2 26
PerCPeF710	CD161	0.5 6.5
Y590 PE		
Y615 PE Dazzle		
Y780 PE	CD3	0.5 6.5
Vio770		
R670 APC		
R780	CD8	1.5 19.5
APCFire750	PBS	11.2 145.6
20.5 μl/rxn;		

Tetramer Test B1	Ex vivo MAIT-tet	Surface μL x
V450 BV421		-
V525 BV510	Aqua L/D	1:500 -
V670 BV650		-
B530 FITC	CD4	1.8 23.4
B710	CD45RA	2 26
PerCPeF710	CD161	0.5 6.5
Y590 PE		
Y615 PE Dazzle		
Y780 PE	CD3	0.5 6.5
Vio770		
R670 APC	(3) Tetramer (1,2,5)	
R780	CD8	1.5 19.5
APCFire750	PBS	11.2 145.6
20.5 μl/rxn;		

Thaw start @ 4:10 pm  
45 stain @ 4:44  
Count @ 5:00 pm  
29.4 million cells  
225 50 @ 1M, not 1.5M  
24.4 sds:

Cells spin @ 5:28 pm  
L/D @ 5:48 pm  
Sds @ 5:56 pm  
6:25 add Tets  
→ 6:37  
6:40 →  
ACDId sds 1:500, 1:1000  
short on ab  
Fit = 10pl, 2 1:500 little more  
Same thing CD4-57  
APC 1:200 2 APC  
Sds 1:200

6:50 pm ugh .... shift ....

6:45 + 20 = 7:05 pm  
[7:07]

Extra repeats @ 7:21 pm  
Done @ 7:36 pm

Tet: 6:25 → 6:37  
25 min 6:50 13 min  
+17 7:07 +17  
42 min 30 min

+14 minutes for  
4 machine tubes

1:200 1:500 1:1000

5 2 1

1pl → 9pl PBS = 10pl

2x8 = 25  
x 900  
20000 → 90pl

50 ~ 70 pl after dump bbt /  
+ 20 pl ≈ 1:100 vortex





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Concurrent Staining (20 minutes, 4°C)

Tetramer Test A1	Ex vivo MAIT-tet	Surface $\mu\text{L}$ x	
V450 BV421	CD4	1.8	
V525 BV510	Aqua L/D	1:500	
V670 BV650			
B530 Alexa488 (FITC)	6FP/5OP-RU		
B710 PerCPeF710	CD45RA	2	
Y590 PE	CD161	0.5	
Y615 PE Dazzle			
Y780 PE Vio770	CD3	0.5	
R670 APC			
R780 APCFire750	CD8	1.5	
20.5 $\mu\text{L}/\text{rxn}$ ;	PBS		

Tetramer Test B1	Ex vivo MAIT-tet	Surface $\mu\text{L}$ x	
V450 BV421			
V525 BV510	Aqua L/D	1:500	
V670 BV650			
B530 FITC	CD4	1.8	
B710 PerCPeF710	CD45RA	2	
Y590 PE	CD161	0.5	
Y615 PE Dazzle			
Y780 PE Vio770	CD3	0.5	
R670 APC	6FP/5OP-RU		
R780 APCFire750	CD8	1.5	
20.5 $\mu\text{L}/\text{rxn}$ ;	PBS		

Separate Staining (30 minutes tetramer RT, 20 minutes 4°C) (postponed)

Tetramer Test A2	Ex vivo MAIT-tet	Surface $\mu\text{L}$ x	
V450 BV421	CD4	1.8	
V525 BV510	Aqua L/D	1:500	
V670 BV650			
B530 Alexa488 (FITC)	6FP/5OP-RU		
B710 PerCPeF710	CD45RA	2	
Y590 PE	CD161	0.5	
Y615 PE Dazzle			
Y780 PE Vio770	CD3	0.5	
R670 APC			
R780 APCFire750	CD8	1.5	
20.5 $\mu\text{L}/\text{rxn}$ ;	PBS		

Tetramer Test B2	Ex vivo MAIT-tet	Surface $\mu\text{L}$ x	
V450 BV421			
V525 BV510	Aqua L/D	1:500	
V670 BV650			
B530 FITC	CD4	1.8	
B710 PerCPeF710	CD45RA	2	
Y590 PE	CD161	0.5	
Y615 PE Dazzle			
Y780 PE Vio770	CD3	0.5	
R670 APC	6FP/5OP-RU		
R780 APCFire750	CD8	1.5	
20.5 $\mu\text{L}/\text{rxn}$ ;	PBS		

## Compensation Notes:

MAIT APC OP-RU looks great after compensation

★ tetramers blocking CD8? (vs CD45RA★)

- CD3 blobs vs CD45RA

★ hCD1d tetramer  $\frac{1}{10}$  blob.  $\swarrow$  placed @ 8?  
(not happening with OP-RU)

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Concurrent Staining (20 minutes, 4°C)

Tetramer Test A1	Ex vivo NKT-tet	Surface $\mu\text{L}$ x	
V450 BV421	CD4	1.8	
V525 BV510	Aqua L/D	1:500	
V670 BV650			
B530 FITC	hCD1d/slo		
B710 PerCPeF710	CD45RA	2	
Y590 PE	CD161	0.5	
Y615 PE Dazzle			
Y780 PE Vio770	CD3	0.5	
R670 APC			
R780 APCFire750	CD8	1.5	
20.5 $\mu\text{L}/\text{rxn}$ ;	PBS		

Tetramer Test B1	Ex vivo NKT-tet	Surface $\mu\text{L}$ x	
V450 BV421			
V525 BV510	Aqua L/D	1:500	
V670 BV650			
B530 FITC	CD4	1.8	
B710 PerCPeF710	CD45RA	2	
Y590 PE	CD161	0.5	
Y615 PE Dazzle			
Y780 PE Vio770	CD3	0.5	
R670 APC	hCD1d/slo		
R780 APCFire750	CD8	1.5	
20.5 $\mu\text{L}/\text{rxn}$ ;	PBS		

Separate Staining (20 minutes tetramer RT, 20 minutes 4°C) postponed //

Separate Test A2	Ex vivo NKT-tet	Surface $\mu\text{L}$ x	
V450 BV421	CD4	1.8	
V525 BV510	Aqua L/D	1:500	
V670 BV650			
B530 FITC	hCD1d		
B710 PerCPeF710	CD45RA	2	
Y590 PE	CD161	0.5	
Y615 PE Dazzle			
Y780 PE Vio770	CD3	0.5	
R670 APC			
R780 APCFire750	CD8	1.5	
20.5 $\mu\text{L}/\text{rxn}$ ;	PBS		

Separate Test B2	Ex vivo NKT-tet	Surface $\mu\text{L}$ x	
V450 BV421			
V525 BV510	Aqua L/D	1:500	
V670 BV650			
B530 FITC	CD4	1.8	
B710 PerCPeF710	CD45RA	2	
Y590 PE	CD161	0.5	
Y615 PE Dazzle			
Y780 PE Vio770	CD3	0.5	
R670 APC	hCD1d		
R780 APCFire750	CD8	1.5	
20.5 $\mu\text{L}/\text{rxn}$ ;	PBS		



## LSR-II log:

- gate naming / gate resetting took ~ 35 minutes
- compensation relatively clean, BM21/S10 1 degree overlap  
KIS directly on Y590
- avoided the outer edge of histogram (red autofluorescence).

- started running Mult P1a @ 10:30, clean.
- spilling intake fluid

**\* Instituting a 5 minute cutoff \***

$$\begin{array}{r} 3 \\ 24 \\ \times 7 \\ \hline 168 \end{array} \quad \begin{array}{r} 24 \\ \times 6 \\ \hline 144 \end{array} \quad \begin{array}{|c|} \hline 24 \\ \hline \times 5 \\ \hline 120 \\ \hline \end{array}$$

~~Fok~~ MATISOP-RV MRA still having pop.

\*  $\downarrow$  concentration doesn't seem to be effective\*  
but non-specific specific is gone.


$$\begin{array}{r} 15 \\ \times 6 \\ \hline 90 \end{array} + 11:16 \checkmark$$

GFP moved FSC back from 570  $\rightarrow$  560  
1:200 messy, 1:500 clean. Set of CD3

Werner Fok NKT height looks better vs other one. {increase to 6 minutes run time}  
1:500 lot better...  $\uparrow$  to characterize the population better

$\hat{=}$  Alternate gating strategy?  
needed sole 1:500 messy.

\* PR670 is compensation driven? \*

APC Nat  better, but not quite @ 1:200  $\checkmark$   
bull at this w/ Zebra contours to differentiate. ~ 5 minutes each

\* APC - GFP shortening to 3 minutes.  $\uparrow$  does seem to be a there/not character

APC cld CD4 trail... concerning.

\* cld 1:1000 ran out of pressure, still sheathe fluid inside, just not acquiring  
(or I forgot vortex?)

Done @ ~~12:55~~ 12:55 pm