

August 13-14, 2021

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

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+M	Total	1.0E+6	2E+6
NY062A	Adult	Berenson	20EG	08/12/21	40 RT, 40°C 40 RT + 20°C	4 ml	3.81E6	5.36E6	15.24	2624	784
NY062B	Adult	Water + Biozox	20EG	08/12/21	for cell & tetramers + Plc MAIT + Plc NKT	4 ml	3.63E6	4.93E6	14.52	2754	824
									29.76	→ 26M - 2 (24) (8)	

Reference - Actual MAITs

	Ex vivo MAIT-pla	Surface $\mu\text{L}$ x 1
V450 BV421	Va7.2	2
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		
R780 APCFire750	CD8	1.5
20.5 $\mu\text{L}/\text{rxn}$	PBS	12.2

5  
27 → - 40  
x 3 → 43.24

21.6  
ml

26  
x 3  
78  
→ 8 (4/20)  
86

Tetramer Test A1	Ex vivo FITC	Surface $\mu\text{L}$ x 12.5
V450 BV421	CD4	1.8 22.5
V525 BV510	Aqua L/D	1:500
V670 BV650		
B530 Alexa488 (FITC)	Tetramer	
B710 PerCPeF710	CD45RA	2 25
Y590 PE	CD161	0.5 6.25
Y615 PE Dazzle		
Y780 PE Vio770	CD3	0.5 6.25
R670 APC		
R780 APCFire750	CD8	1.5 18.75
20.5 $\mu\text{L}/\text{rxn}$	PBS	14.2 177.5

11:30 AM

NY062 frozen upside down

Spec & unthawed when inserted upside down → thaw

spec B f → water bath

Thawed into 9ml per R10 vs 7 (since closer 20)

11:45am - stain in

\* count start @ 11:56 am \*

\* clog \* Quick clean...  
dfs! Dues 66

12:03 start...

Reference - Actual NKTs

	Ex vivo NKT-pla	Surface $\mu\text{L}$ x 1
V450 BV421	Va24ja18	2
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		
R780 APCFire750	CD8	1.5
20.5 $\mu\text{L}/\text{rxn}$	PBS	12.2

Tetramer Test B1	Ex vivo APC	Surface $\mu\text{L}$ x 12.5
V450 BV421		
V525 BV510	Aqua L/D	1:500
V670 BV650		
B530 FITC	CD4	1.8 22.5
B710 PerCPeF710	CD45RA	2 25
Y590 PE	CD161	0.5 6.25
Y615 PE Dazzle		
Y780 PE Vio770	CD3	0.5 6.25
R670 APC	Tetramer	
R780 APCFire750	CD8	1.5 18.75
20.5 $\mu\text{L}/\text{rxn}$	PBS	14.2 177.5

12:28 ab prep start

ABs done @ 1:02pm

1.3) → cells spinning down, clumps forming just enough for all tubes

Now out 10% PBS shed

AP hCD1d GFP

40 min 4°C

4:02:53 - 4:00:00 4°C

5c's @ 14:06pm

2:18 4°C spin down

For continued timing →

40 min @ RT 4

40 min @ 4°C 4

20 min RT + 20 min @ 20°C 4

20 min @ 4°C + 20 min @ 20°C 4

40 min @ RT + 20 min 4

40 min @ 4°C + 20 min 4

55% tetramer

↑ 6 x 2 pl = 12  
all same time

2 + 12

PIA Mat/WRT → 2:32 pm + 20 = 52

Sols D3U 2:34 pm

⚡ F! @ 2:38 Ate OPRoc max, switched labels

40 min RT + 20 min @ 2:41 pm → 3:21 pm

tetramers in @ 2:47 pm

40 min RT in @ 2:49 pm → 3:29 pm

40 min 4°C in @ 2:51 pm → 3:31 pm

→ 20 min @ 4°C @ - 3:37 → 3:57 pm

Done @ 4:11 pm

Mat OPRoc/GFP

1:200

5 pl (0.00)

MT hcdid/sls →

1:500

2 pl (1:10)

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

% dp ~ decoupling tetramers?

Phen  
CP-RV  
heptid  
IP  
heptid  
IP solo

stim  
heptid  
IP  
CP-RV  
FP

will stim change binding?

vs  
will stim change neg controls background?



Flow Run 08/16/2021 @ 7:12 pm

Water running clean ✓

~ 130 euts/sec 5000 ~ 30 secs

APC turned ditto APC Am

& BUGSO ↓ all good beyond this.

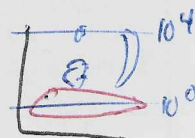
FSC up to 580. ~ 1500 euts/sec. P1a Maint. 6 1/2 min

Va24 & CD61 ~ high Va24 CD61 high.

very relatively few for this individual?

Pop low stamers.

(2 µl?) ← may need to titrate.



Maint start @ 7:41 pm Fite ≠ RT, < 4°C (distinguishable) ← Separated ✓

→ CD8 intermediate.

200 ~ tetramer?

↑ looks really good.

24  
x 6.5  
120  
1440  
156 → 2 1/2

\* APC OP-RO 40°C → dump (prime removal). run low. cleaner dump vs 40 RT.  
work even better w/ separate stirring

Net's ≠ CD61 ±, spread? ± ...  
Fite 40 RT < 40°C (less contam?)

20°C

APC 40°C nice on fire comp

LIUPT + 20°C

loss non-specific?

(very few events @ 2 min ...)

77 euts only clean dump  
(200 euts)

2nd half: 09:04 pm

5 minutes for each 12 12 12  
5 5 5  
negative control (vs 6 1/2 for actives)

RT 4°C separate

\* gradient cleanliness \*

103 Monday →  
"Infant data"  
"track a sample"

Done @ 10:20 pm ~ 3 hrs for 20 samples + comp  
(no setup label time)