August 23 , 2021

HEU: Innate-like T cells
Mixing Tetramers & Biotin

[Logged over from PHB]

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+M	Total	1.5E+6	2E+6
ND006						3	3.78E4			265µl	
ND006						3	3.93E4			254µl	
CQ1070 #8611						3	5.3 E6			189µl	

[6 tubes]

Tetramer Test	Ex vivo Both	Surface µL	x 6.5
V450 BV421	CD4	1.8	11.7
V525 BV510	Aqua L/D	1:500	-
V670 BV650			
B530 Alexa488 (FITC)	hCD1d Tetramer		
B710 PerCPeF710	CD45RA	2	13
Y590 PE	CD161	0.5	3.3
Y615 PE Dazzle			
Y780 PE Vio770	CD3	0.5	3.3
R670 APC	MR1 Tetramer		
R780 APCFire750	CD8	1.5	9.75
20.5 µl/rxn;	PBS	11.2	72.8

← 2µl
← leaving these out master mix
in 3
17.5
← 5µl

★(17.5 draw)★

2 draws each Fitc hCD1d/solo
12 each APC MR1/OPRU/6FP
x 5
60µl

6µl tet into 54 (1:10)

[92M]

	Pcb	comb	PE's	biot	cont	cont tets		
Aqua	1	6	-	6	2	4	= 19	x 800 = 15,200
Horizon	3	-	6	-	-	-	= 9	

7
x 4 [?]
152

Horizon → 9µl in 9 mls ~D h: 12:26
30.4µl in 15.2mls ~/D Aqua@12:31

32
x 3
106

LSR - messed up CD3 FSC gate (CLD misfeed)

Tetramer Test	Ex vivo Both	Surface µL	x
V450 BV421	CD4	1.8	
V525 BV510	Aqua L/D	1:500	
V670 BV650			
B530 Alexa488 (FITC)	hCD1d Tetramer		
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 µl/rxn;	PBS		

Tetramer Test	Ex vivo Both	Surface µL	x
V450 BV421	CD4	1.8	
V525 BV510	Aqua L/D	1:500	
V670 BV650			
B530 Alexa488 (FITC)	-		
B710 PerCPeF710	CD45RA	2	
Y590 PE	CD161	0.5	
Y615 PE Dazzle			
Y780 PE Vio770	CD3	0.5	
R670 APC	MR1 Tetramer		
R780 APCFire750	CD8	1.5	
20.5 µl/rxn;	PBS		

[Done @ 4:50pm]

Tetramers ✓

Mixed (or no tetramers)

deal CD26 separately

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~~Mixing Tetramers & Biotin~~ NKT P2 +/- CCR7
W/ NKG2D ~ biotin ~ non-biotin

{6 tubes?}

mastermix

Vα Test A	Ex vivo NKT-p2	Surface µL x 1		Intra µL	x 4.5
BV421	Va24	2			
BV510	CD3	1.5			
BV650	NKG2D Streptavidin	2 (1.5)			
Alexa 488 (FITC)	dg9	-		3	13.5
PerCPeF710	CD56	1			
PE	PD1	1.5			
PE Dazzle					
PE Vio770	NKG2A	0.6			
APC	CCR6	2.5			
APC Fire750	Horizon L/D	1:1000			
20.5 µl/rxn;	PBS	9.4		18.5	83.3

Not the [illegible]

Vα Test B	Ex vivo NKT-p2	Surface µL x 1		Intra µL	x
BV421	Va24	2			
BV510	CD3	1.5			
BV650	NKG2D Streptavidin	2 (1.5)			
Alexa 488 (FITC)	dg9	-		3	
PerCPeF710	CD56	1			
PE	PD1	1.5			
PE Dazzle	CCR7	1.5			
PE Vio770	NKG2A	0.6			
APC	CCR6	2.5			
APC Fire750	Horizon L/D	1:1000			
20.5 µl/rxn;	PBS	7.9			

☆ ...I added right.

18.5
× 4.5
925
7400
83.25

Missing? 2!

2!

Tetramer A	Ex vivo NKT-p2	Surface µL x 1		Intra µL	x
BV421	~~VD2~~ ccr7	[illegible]			
BV510	CD3	1.5			
BV650	NKG2D? ☆ fused	1.0 ↓			
Alexa 488 (FITC)	dg9	-		3	
PerCPeF710	CD56	1			
PE	PD1	1.5			
PE Dazzle	☆ CCR7 ☆	1.5			
PE Vio770	NKG2A	0.6			
APC	hCD1d PBS-57/solo				
APC Fire750	Horizon L/D	1:1000			
20.5 µl/rxn;	PBS	14.9			

brou, down cone

Tetramer B	Ex vivo NKT-p2	Surface µL x 1		Intra µL	x
BV421	~~VD2~~ ccr7	1.5			
BV510	CD3	1.5			
BV650	NKG2D? * fused	1.0 ↓			
Alexa 488 (FITC)	dg9	-		3	
PerCPeF710	CD56	1			
PE	PD1	1.5			
PE Dazzle	[illegible]				
PE Vio770	NKG2A	0.6			
APC	hCD1d PBS-57/solo				
APC Fire750	Horizon L/D	1:1000			
20.5 µl/rxn;	PBS	13.4			

~~RBC lyse~~

Short 2 aliquots

Cant remember if I grabbed biotin NKG2D or CD26... I think safe? well find out...

☆ Healthy Vα24 staining vs CCR6 in this panel.
☆ FSC off because you fixed permed these, switched 5900n [illegible]
lot dead cells in solo A tet

Everything is messy as hell.

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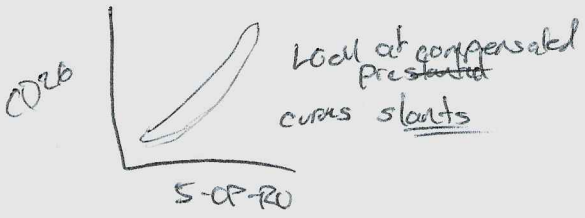
1) Tetramer 40 min RT, wash, Abs 20 min 4*C wash, Streptavidin 15 minutes 4*C, Wash
2) Tetramer + Abs 20 min 4*C, Strep 15 min [known horror]
3) CD26 + Strep, Wash, Tetramer 40 min RT, wash, ~~Abs 20 min 4*C wash~~

abs

↳ could see if ~~flipped~~ original way maybe working too.

Tetramer Test B1	Ex vivo APC	Surface µL	x 11
V450 BV421	CCR6	2.5	27.5 → 22 (5.5 pBS)
V525 BV510	Aqua L/D	1:500	
V670 BV650	~~CD26 - Strep~~		
B530 FITC	CD4	1.8	19.8
B710 PerCPeF710	CD45RA	2	22
Y590 PE	CD161	0.5	5.5
Y615 PE Dazzle	CCR7	1.5	16.5
Y780 PE Vio770	CD3	0.5	5.5
R670 APC	Tetramer (+/-)		(5) 15.5
R780 APCFire750	CD8	1.5	16.5
20.5 µl/rxn;	PBS	~~5.2~~	57.2

← yes was done
← Lewis was at mastermix w/ APC-mart

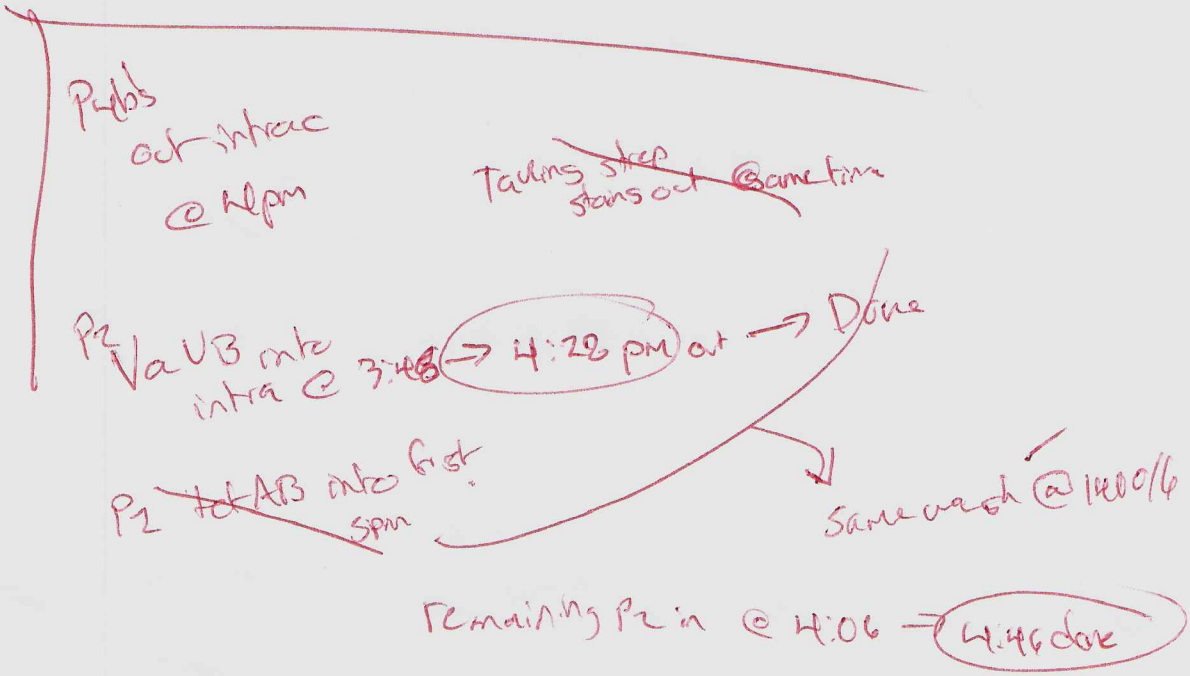


May not be as distinguishable since MAITs are robust...

~~[illegible] drawn~~
(~~15.5 drawn~~)
(15.5 drawn)

2 ml washes in between Strep containing steps

300
x 8
3000



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APC ~~MAIT [20 min 4*C] vs [40 min RT + 20 min 4*C] *{4 tubes}*~~

Tetramer Test B1	Ex vivo APC	Surface µL	x
V450 BV421	CCR6	2.5	
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	CCR7	1.5	
Y780 PE Vio770	CD3	0.5	
R670 APC	Tetramer		
R780 APCFire750	CD8	1.5	
20.5 µl/rxn;	PBS		

[postponed]

☆ APC OP-RU/6FP in cord

☆ APC hCD1d/Sdo in cord

— baseline characterization. (abs ✓ just more tetramer....

☆ order more CD3 BV510 ☆
☆ CCR6 BV421 ≈ APC caps very similar!!

LSR 08/24/21 ⇒ One hour to setup a run Dazzle comps?!

N0006 MAITs controls ≈ 1% CD3 CCR6+ CD45RA− CCR7− CD8

NKT staining ⇐ CD161, real, wrong marker? TBD...

OR-RU CQ ugly OP-RU smear on CD3− pop ... my tandems w/ BV650? nope on APC after collection graph cancelled.

None on hCD1d, visible population.... mistaken tetramers? just compensation is shifted. Few CD4s.

	Va	2
	L/D	Aqua
	—	
	CD4	1.8
	CD45RA	2
PE	CD161	0.5
	CCR7	1.5
PerCP	CD3	0.5
APC	CCR6	2.5
	CD8	1.5
	PBS	8.2

Single Va24

Va7.2

mix controls

~~add in CD24...?~~

after surface stain ... execute decision