July 23-24th, 2021

HEU: P2 γδ Cord + MAIT Panels Experiment #1

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+M	Total	1.5E+6	2E+6
*8154 CQ644						~2ml	4.5E6	6E+6	9.0million	.333µl	~6wells
NY44						800µl	7.5E6	13E6	6million	~40µl x 30 diff cells sc's	.30µl <c= 2.7M ~

300 ~2 days for 4 Enough for P2's

~245µl/ 2wells for Mait's

	Ex vivo MAIT-p1a	Surface µL x 1
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 ← Lot to lot amount
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 µl/rxn;	PBS	7.2µl

Tetramer Test A 1:500	Ex vivo MAIT-p1a	Surface µL x 1
V450 BV421	CD4	1.8
V525 BV510	Aqua L/D	1:500
V670 BV650	Strep	1
	CD26	1
B530 Alexa488 (FITC)	~~6FP-~~ 5OP-RU	2µl (1:100) ← 1.5 mg/ml ... 1:500
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 µl/rxn;	PBS	7.2µl

EP2Δ

Mait Pos Mait for PinFits

+11 sc's.

Staining Volume: 100µl

1:100 = 1µl

1:500 = 0.2µl

Dilution 1:100 → 1µl + 9µl↑

10µl → (2µl) ✓

FBS

6x3=18

2x4=8

~ 30/15ml's

5x8 = 40ml → 8µl 5/0 Aqua

3 ml - 3µl horizon

6x3=18

2.18

RBC lysis ~~pellet~~ 1-9ml

6·300 = 800µl

1:10 so...

48392 R 54063 hMR1 5-OP-RU Alexa488 1.5 mg/ml 2021-02

1.0µl → 9µl:

Need to make own stock FBS.

CD45RA mait panels #1 100 → surface GRIZZ → Foxperm

02/24/2021

1:05pm → Panels setup on software

(2) 13:46 strep - FBS wash (2mls) (1) scs made @ 11:38am

(1x) wash enough or ~~2x~~ ...

TBD ~~#~~ → Fixing PFA - PBS ...

Resuspend samples in ~~200µl~~ ...

~ 1½ run time this weekend ...

2:00 pm tetramers done.

Spun @ 11:55am

Lyse @ 12:16 pm

Single colors @ 12:[illegible] 26 pm

12:52 Surface stain

scs done 1:08pm

Intracell start 14:15 pm → 2:55 pm

Spun @ 2:53pm (due to Carryover)

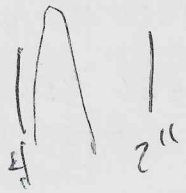
13:18 cells post RBC lysis in. Mait sitting + 5 minutes before added FBS-PBS.

Mait strep @ 13:31 4°C 15min → 746

P2 Cytfix/Perm @ 1:33pm

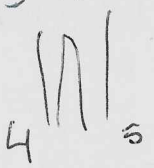
Mat control setting

Y615



Pe.

Y590



Y615

similar story 450-525

Mat Pin - 510 FSC.

... as soon as collected graphs reverted to squished

- - - -

t 1:500 what pop?

July 23-24th, 2021

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Makes 6 stock

June	Ex vivo Gd-p2	Surface µL x 1		Intra µL x 6.5	
BV421	VD2	1			
BV510	Aqua L/D	1:500			
BV650	NKG2D	2µl			
Alexa 488 (FITC)	dg9	-		3	19.5
PerCPeF710	CD3	1.5			
PE	PD1	1.5			
PE Dazzle					
PE Vio770	NKG2A	0.6			
APC	VD1	1.0			
APC Fire750					
20.5 µl/rxn;	PBS	12.9		17.5	113.8

June	Ex vivo Gd-p2	Surface µL x 1		Intra µL x 1	
BV421	VD2	1			
BV510					
BV650	NKG2D	2			
Alexa 488 (FITC)	dg9	-		3	
PerCPeF710	CD3	1.5			
PE	PD1	1.5			
PE Dazzle					
PE Vio770	NKG2A	0.6			
APC	VD1	1.0			
APC Fire750	Horizon L/D	1:1000			
20.5 µl/rxn;	PBS	12.9		17.5	

June	Ex vivo Gd-p2	Surface µL x 1		Intra µL x 1	
BV421	VD2	1			
BV510	Aqua L/D	1:500			
BV650	NKG2D	2			
Alexa 488 (FITC)	dg9	-		3	
PerCPeF710	CD3	1.5			
PE	PD1	1.5			
PE Dazzle					
PE Vio770	NKG2A	0.6			
APC	VD1	1.0			
APC Fire750	CD16	1			
20.5 µl/rxn;	PBS	11.9		17.5	

June	Ex vivo Gd-p2	Surface µL x 1		Intra µL x 1	
BV421	VD2	1			
BV510	CD3	1.5			
BV650	NKG2D	2			
Alexa 488 (FITC)	dg9	-		3	
PerCPeF710	CD16	1.0			
PE	PD1	1.5			
PE Dazzle					
PE Vio770	NKG2A	0.6			
APC	VD1	1.0			
APC Fire750	Horizon L/D	1:1000			
20.5 µl/rxn;	PBS	13.4		17.5	

June	Ex vivo Gd-p2	Surface µL x 1		Intra µL x 1	
BV421	VD2	1			
BV510	Aqua L/D	1:500			
BV650	NKG2D	2			
Alexa 488 (FITC)	dg9	-		3	
PerCPeF710	CD3	1.5			
PE	PD1	1.5			
PE Dazzle					
PE Vio770	NKG2A	0.6			
APC	VD1	1.0			
APC Fire750	CD56	1.0			
20.5 µl/rxn;	PBS	11.9		17.5	

June	Ex vivo Gd-p2	Surface µL x 1		Intra µL x 1	
BV421	VD2	1			
BV510	CD3	1.5			
BV650	NKG2D	2			
Alexa 488 (FITC)	dg9	-		3	
PerCPeF710	CD56	1.0			
PE	PD1	1.5			
PE Dazzle					
PE Vio770	NKG2A	0.6			
APC	VD1	1.0			
APC Fire750	Horizon L/D	1:1000			
20.5 µl/rxn;	PBS	13.4		17.5	

P2 Adjusted R620 / R780 up. Not much <> [illegible]

R780 pretty low. BUH21 + PE really high voltage gate

V450 V510 very close as well

ditto PE Pdazzle.

gate U670 huge! (reset?)

* (Started running panel 2 @ 1:14 (so 42:17) *

Switched FSC up to 530* For Aqua A

~ running around 5000 evts/sec.

resuspended in 200µl ?
~ 7 minutes

Aqua A - NK62D squished (U670), similar for dg9 + PD1? (B530, Y590)
both upped on scale ($10^3 - 10^5$)

NK62A Y780 off map ← L/D balls messy 525.

Aqua B - VD2 (450) ~~||||~~ at 10^0 vs CD3
squished NK62D

moved FSC back to 520

? should've set L/D Δ comp for Aqua/Horizon?

* - Y590 PD1 + CD16 showing stringers * + NK62A

Aqua C: NK62D squish. CD56 blur.

Horizon A - very clean : NK62D not as squished

Horizon B → dg9 off comp (B530) CD16 also off (B710)
→ weird clash ~~on VD2~~ ~~CD3~~ - cells. stringers, ...

Try other clone?

Horizon C → B530 450 clash? B710
↖ compensation off.

VD2 // not good...