

November 15-16th, 2021

HEU: Ex-vivo $\gamma\delta$ CBMC panels #11 & ILT novo 4

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	2E+6	1.5E+6
Inf179										372 μ l	
Inf191										257 μ l	
NY062										386 μ l	

RNA in @ 11:18 AM

ooo I forgot unstained!

^ (after I trized everything)

$\gamma\delta$ NK1 ccr7
 OO OO OOOO
 OO OO
 OO OO

6:46 pm tetamers \checkmark \rightarrow 7:26
Spin @ 7:30

Abs prepped @ 7:39

37°C inc 7:46 \rightarrow 8:16 pm out
@ 8:30 40°C

~~spin~~ Rbc \rightarrow PFA for 3 tubes, grazus!

Rbc lyse @ 9:11 pm / +15 min, ugh...
Spin @ 9:17 pm + NO FBS ready, thanks Nages...

Qc -fail UV3 6.82%

Fx Perm @ 9:27 pm Indra @ 22:21

NK1 Inf179 cu-ctrl = PFA fixed accidents And it worked....

Inf191 cu-ctrl

+ NK1 inf179 cu PMA

Oh... it was NY062

[screenshot worked]

Don't forget unstained in the future!

Ny062 is messy vs its MAITS.

$\frac{10}{8} \times 8$ $\frac{10}{12} \times 1.2$
 $\frac{10}{5} \times 5$ $\frac{10}{50\mu l}$ 2-18, need GMR1 6-54

I increased my threshold to 250,000

#	Filter	Single color (u)	Ref cft	Fluorochrome	Marker	During stimulation	Tetramer (RT) for 40 min	37°C (RT) for 30min	Surface staining 40°C for 30min	Lysing then Cytofix/Perm	RT for 40min Universal Intra mix (10x diluted)
1	UV2	1.25	Cells	C	BUV395 CD62L (DREG-56)				1.2	12.0	
2	UV7	1.25	Cells	C	BUV496 CD8 (RPA-18)				1.0	10.0	0.25
3	UV9	1.25	Cells	C	BUV563 CD69 (FNSO)				1.0	10.0	0.25
4	UV10	1.25	Cells	C	BUV615 CCR4 (IG1)			2.0	20		2.5
5	UV11	1.25	Cells	C	BUV661 Vβ2 (B6)				1.0	10.0	
6	U14	1.25	Cells	C	BUV737 CXCR3 (16/CXCR3)			2.5	25		
7	UV16	1.25	Cells	C	BUV805 CD4 (SK3)				1.5	15.0	0.25
8	V1	1.25	Cells	C	BUV421 CD127 (A019D5)	0.8			1.0	10.0	2.5
9	V3	1.25	Cells	C	Pacific Blue CD14 (M512)				2.0	20.0	
10	V5	1.25	Cells	C	Pacific Blue CD19 (H1B19)				2.0	20.0	
11	V7	1.25	Cells	C	BUV480 CD161 (R6A631)	0.8		1	10		0.5
12	V10	1.25	Cells	C	BUV510 (dIm) CD45RA (H100)				1.0	10.0	5
	V11	-	Cells	C	BUV605 CD56				1.0	10.0	
28	V13	-	Cells	C	BUV500 Hsu CCR7 (separately)	0.8			1.0	10.0	
13	V14	1.25	Cells	C	BUV711 David CCR7 (separately)						
14	V15	1.25	Cells	C	BUV750 JFNAE (B27)						1.5
15	B2	1.25	Cells	C	BUV786 CCR6 (11A9)			1.8	18		15
16	B3	1.25	Cells	C	Alexa488 hCD1d		<1:750>				
17	B4	1.25	Cells	C	Sparkblue 550 CD2 (SK7)				1.2	12.0	0.25
18	B6	1.25	Cells	C	PE NK62D				1.0	10.0	2.5
19	B8	1.25	Cells	C	PE-CF594 CD26 (M-A261)			1.2	12		
20	B10	1.5 (<10^4)	Cells	C	PE-Cy5 CD25 (M-A251)				1.2	12.0	0.25
21	B13	-	Cells	C	PerCP-Cy5.5 JNFA (MAB11)						2.5
22	R1	1.25	Cells	C	PE-Mo770 PD1				1.5	15.0	
23	R2	1.25	Cells	C	APC Alexa Fluor 647 hMβ1 50P-RU						1.5
24	R4	1.25	Cells	C	APC-R700 CD107a (H4A3)	6.0	<1:500>				
25	R6	1.2500	Cell	P	Zombie NIR Viability						
26	R7	1.3	Cells	P	APC/hTie 750 CD27 (O323)			2	20		
27	R8	1.3	Cells	C	APC/hTie 810 CD38 (HT2)			1.6	16		
Total volume							8.4	12.1	18.6	7.25	
unstained Cells											
24 color CBMC											

Brilliant Stain: 50 50 50
 Draw Volume: 57.1 63.6 52.3

587
 65 (H4A3)
 RT
 RT
 RT

Protocol

Aliquot cells	
PMA-ionomycin stimulation	
Golg blockers	
PBS 2ml	1400rpm, 6min
Live/Dead @ RT 15min	
PBS-FBS 2ml	1400rpm, 6min
Tetramers @ RT 40min	
PBS-FBS 2ml	1400rpm, 6min
Surface	CD4 CD26
37°C 30min	CXCR3 CD27
	CD161 CD38
PBS-FBS 2ml	
Surface mix @ 40°C 30min	1400rpm, 6min
Lysing solution 300-500ul	
PBS-FBS 2ml	1400rpm, 6min
Cytofix/perm @ 40°C 20min	
wash twice PermWash 1 ml	1500rpm, 6min
Intra staining @ RT for 40min	
wash once w/ 2ml	1500rpm, 6min
Resuspend 100 ul PFA-PBS	

#	Filter	Single color (u)	Ref ctrl	Fluorochrome	Marker	During stimulation	Tetramer (RT) for 40 min	37°C (RT) for 30min	Surface staining 40c for 30min	Lysing, then Cytofix/Perm	RT for 40min Universal Intra mix (10x diluted)		
1	UV2	1.25	C	BUV395	CD62L (PREG-56)				1.2	7.2			
2	UV7	1.25	C	BUV496	GD8 (RPA-18)				1.0	6.0	0.25		
3	UV9	1.25	C	BUV563	CD69 (FEN50)				1.0	6.0	0.25		
4	UV10	1.25	C	BUV615	CCR4 (1G1)			2.0					
5	UV11	1.25	C	BUV661	Vβ2 (B6)				1.0	6.0			
6	UV14	1.25	C	BUV737	CD38 (1G6/CD38)			2.5					
7	UV16	1.25	C	BUV805	CD4 (SK3)				15				
8	V1	1.25	C	BUV421	CD127 (A019P5)	0.8			1.5	9.0	0.25		
9	V3	1.25	C	Pacific Blue	CD14 (M5E2)				1.0	6.0			
10	V5	1.25	C	Pacific Blue	CD19 (HB19)				2.0	12.0			
11	V7	1.25	C	BUV480	CD161 (REA631)	0.8		1	6		0.5		
12	V10	-	C	BUV510 (dim)	CD45RA (HI100)				1.0	6.0			
13	V11	-	C	BUV605	CD56	0.8			1.0	6.0			
14	V13	-	C	BUV711	Hsu				1.0	6.0			
15	V14	1.25	C	BUV750	IFNγ (B27)						1.5		
16	B2	1.25	C	BUV786	CCR6 (11A9)			1.8	10.8		9		
17	B3	1.25	C	Alexa488	hCD1d		<1:750>						
18	B4	1.25	C	Spark blue 550	CD3 (SK7)				1.2	7.2	0.25		
19	B6	1.25	C	PE	NK62D				1.0		1.5		
20	B8	1.25	C	PE-CF594	CD26 (M-A261)			1.2	7.2				
21	B10	1.5 (<10x4)	C	PE-Cy5	CD25 (M-A251)				1.2	7.2	0.25		
22	B13	-	C	PerCP-Cy5.5	INRA (MAB11)						2.5		
23	R1	1.25	C	PE-σ6770	PD1				1.5		15		
24	R2	1.25	C	APC	IL-4								
25	R4	1.25	C	Alexa Fluor 647	hMR150P-RU						1.5		
26	R6	1:2500	C	APC-R700	CD107a (H4A3)	6.0	<1:500>				9		
27	R7	1.3	C	Zombie NIR	Viability			2	12				
28	R8	1.3	C	APC/Fire 750	CD27 (O323)			1.6	9.6				
29	R8	1.3	C	APC/Fire 810	CD38 (HT2)								
Total volume						8.4	13.1	12.1	72.6	18.6	96.6	7.25	44
unstained Cells													
24 color CBMC													

Brilliant Stain: 50
Draw Volume: 57.1
50
63.6
50
52.3

$$8.9 \leftarrow RLC$$

$$0.8 \times 2 = \frac{1.6}{10.5}$$

$$For each mix$$

Protocol

Aliquot cells	
PMa-Ionomycin stimulation	
Golgi blockers	1400rpm, 6min
PBS 2ml	
Live/Dead @ RT 15min	
PBS-FBS 2ml	1400rpm, 6min
Tetramers @ RT 40min	
PBS-FBS 2ml	1400rpm, 6min
Surface	CCR4 CD26
37°C 30min	CCR3 CD27
	CD161 CD38
PBS-FBS 2ml	1400rpm, 6min
Surface mix @ 40c 30min	
Lysing solution 300-500ul	
PBS-FBS 2ml	1400rpm, 6min
Cytofix/perm @ 40c 20min	
wash twice PermWash 1 ml	1500rpm, 6min
Intra Staining @ RT for 40min	
wash once w/ 2ml	1500rpm, 6min
Resuspend 100 ul PFA-PBS	

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HEU: Ex-vivo $\gamma\delta$ CBMC panels #11

CCR 7's (duplicate infant edition) K4

BV650 CCR7 not made but not etc

BV711 → "IL 4 appears"