October 12-13th, 2021

HEU: Ex-vivo γδ CBMC panels #6 & ILT spectral #3

60 µl
400c/µl
70 µl
700c/µl
60 µl
110 cells/µl
30 µl
260c/µl

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly (1:100)	Ly+Mon	Total	1E+6	1.5E+6
Inf032-5 α-1 (16)	RRFG HU	Box 1A, G8,9		2018-08-03	200µl ·(3.11) = 620M	2.5	3.11	3.8 E6	7.7M (9.5M)		482 µl
Inf156-2 α-1 (7)	S89L HEU-L	Box 5A, H2, 3		2019-01-19	800µl (4.19) = 3.35M	2.5	2.48	3.5 E6	6.2M (8.75)		605 µl
Inf452-1 α-2 (6)	T1J7 HEU-h	Box13A D6-8		2020-12-07	300µl (2.67) = 800M	2.5	2.67	4.3 E6	6.6M (10.75)		562 µl
ND050	Adult		15 E+6/ml	08/24/21		2.5	2.81	4.05E6	7.0M (10.1)	370ul / 1.5E+6 + R10 63ul	142µl

	Ex vivo Gd-p4b	Surface µL	x 3	Intra µL	x 3
BV421	PD1	2	6		
BV510	Aqua L/D	1:500			
BV650	CD56	1.8	5.4		
Alexa 488 (FITC)	Perforin (dG9)	-	-	3	9
PerCPeF710	CD3	1.5	4.5		
PE	GZMb	-	-	1.5	4.5
PE Dazzle					
PE Vio770	NKG2A	0.6	1.8		
APC	Vδ2	1	3		
APC Fire750	CD16	1	3		
20.5 µl/rxn;	PBS	12.6	37.8	16.0	48

	Ex vivo Gd-CK PMA	Surface µL	x 6	Intra µL	x 6
BV421	PD1	2	12		
BV510	Aqua L/D	1:500			
BV650	CD27	1.5	9		
FITC	Vδ2	1.2	7.2	0.2	1.2
PerCPeF710	CD3	1	6	0.2	1.2
PE	CD56	0.5	3		
PE Dazzle					
PE Vio770	IFNγ	-	-	0.6	3.6
Alexa 647 (APC)	TNFα	-	-	2	12
APC Fire750	CD45RO	2	12		
20.5 µl/rxn;	PBS	12.3	73.8	17.5	105

	Ex vivo Gd-p1	Surface µL	x 3
V450 BV421	PD1	2	6
V525 BV510	Aqua L/D	1:500	
V670 BV650	CD16	1.5	4.5
B530 FITC	Vδ2	1.2	3.6
B710 PerCPeF710	CD25	2	6
Y590 PE	CD28	2	6
Y615 PE Dazzle	CD27	1.5	4.5
Y780 PE Vio770	CD3	0.5	1.5
R670 APC	Vδ1	1	3
R780 APCFire750	CD45RA	1.8	5.4
20.5 µl/rxn;	PBS	7	21

	Ex vivo Gd-p2	Surface µL	x 3	Intra µL	x 3
BV421	Vδ2	1	3		
BV510	CD3	1.5	4.5		
BV650	NKG2D	2	6		
	Streptavidin	{1.5}			
Alexa 488 (FITC)	dg9	-		3	9
PerCPVio700	CD56	1	3		
PE	PD1	1.5	4.5		
PE Dazzle					
PE Vio770	NKG2A	0.6	1.8		
APC	Vδ1	1	3		
APC Fire750	Horizon L/D	1:1000			
20.5 µl/rxn;	PBS	11.9	35.7	17.5	52.5

Abs made 7:15-8:24
9:02 thaw start
9:47 stain count
10:02 count start
10:47 PMA in
11:21 L/D
Surface @ 11:57am
strep @ 12:40
12:52pm scs in → 1:07 pm
1:11pm → FixPerm
Intra @ 2:09pm
P2/P4b/CK @ 3:22 pm
NKT: 3:38pm
SCs @ 4:09pm
Tetramer in @ 4:47pm
SC's fix: @ 4:45pm →
L/D @ 5:01
37°C @ 5:13 → 5:43pm

RBC - 20
x.5
10.0 (1:9)

NKT done @ 7:21pm
Done @ 7:54 pm

Surface all @ 5:32
NKT surface @ 6pm
CK RBC lyse @ 6:04pm
CK - FixPerm @ 6:19pm
NKT - RBC lyse @ 6:32pm
NKT fixperm @ 6:46
CK intra @ 7:09pm

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	Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	1E+6	1.5E+6
60µl 380c/µl	Inf32-5 a-2 (16)	RRFH HU			2018-08-03		2.5	3.07	3.69 E6	7.6 M (9.2)	810/3E+6 +190ul	
79µl 880c/µl	Inf156-2 a-2 (7)	589M HEU-L			2019-01-19		2.5	4.19	5.75 E6	10.5 M (14.3)	520/3E+6 +480ul	357µl
69µl 460c/µl	Inf452-1 (6)	HEU-H			2020-12-07		2.5	2.86	4.69 E6	7.15M (11.7)	630ul/3E+6 +370ul	320µl
										ND050	370ul/1.5E+6 +630	

★ Lights not working on LSR-II buttons

PE-CF up 5

scs

R450 ↓ gate

increased 650 by 5 expanded scale

V525 ↑ upper bound Y590 ↑ ↑Y780 gate to [illegible]

V450 – the FSC ≠ cover, adjusted gate

P_1 ⇒ FSC@615 centred med 5200 events.

Y780 CD3 VDI R670 → major off on compensation.

massive spreading error Y780 on p1....

→ adjusted sc gates back, reapplied comp.

→ still present

Y590 & V670

P4b: FSC to 620?

– GranB ≥ 56

Not enough cells for NKT wells, (def not PMA/intrac setup).

– 600K-800K 3 infants ✓.

+ updated + new scs.

+ unstained cells.

★ I think comp #2 was a little bit better

adjusting FSC SSC gate to right clears up some of the spreading error overlap but VDI still falling out.

(mon catching VDI ab or fluo resectly staining aw)

PMA

µl cells	R10	+	
482 +	218		300
605 +	95	+	300
562	138	+	300

925µl + 6µl each

Last P2 tube ↑630 (seems to be hugging cell debris @ 620)

all → keeping @ 630 FSC.

PMA ← ctrl ↔

(drop left @ climb point)

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* Where's my CD8? (repeated issue)
  now it's working somehow...
  - renamed after fact, angry note but so far so good.

CD56 slightly off peak
ditto L/D NIR one below (adjoted)

4:10 pm - dulled save...

Reference controls are spectred first, remember 15 sec before recording
20,000 → 500 events

BUSOS £

The everything is messy & uninterpretable edition.

UMPS → expected to flush done...

Attempt #1 ?

F-Ho CD8 f.x 5-6 BUV496 cells
F-Ho CD163 f.x 5-6 BV711 cells
Zombie NIR Live Dead PBMC
CD27 PBMC

PR-CBMC-CD62L-2021 4/6

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#	Filter	Single color (ul)	Ref ctrl		Fluorochrome	Marker	During stimulation	Live/Dead RT for 15min	Tetramer (RT) for 40 min	37oC (RT) for 30min	x3	Surface staining 4oC for 30min	x3	Lysing, then CytoFix/Perm	RT for 40min Universal Intra mix (10x diluted)	x3
1	UV2	1.25	Cells	C	BUV395	CD62L (DREG-56)						1.2	3.6			0
2	UV7	1.25	Cells	C	BUV496	CD8 (RPA-T8)	•					1.0	3.0		2.5	7.5
3	UV9	1.25	Cells	C	BUV563	CD69 (FN50)						1.0	3.0		2.5	7.5
4	UV10	1.25	Cells	C	BUV615	CCR4 (1G1)				2.0	6					
5	UV11	1.25	Cells	C	BUV661	Vd2 (B6)						1.0	3.0			
6	U14	1.25	Cells	C	BUV737	CXCR3 (1C6/CXCR3)				2.5	7.5					
7	UV16	1.25	Cells	C	BUV805	CD4 (SK3)						1.5	4.5		2.5	7.5
8	V1	1.25	Cells	C	BV421	CD127 (A019D5)	0.8					1.0	3.0			
	V3	1.25	Cells	C	Pacific Blue	CD14 (M5E2)						2.0	6.0			
9	V3	1.25	Cells	C	Pacific Blue	CD19 (HIB19)						2.0	6.0			
10	V5	1.25	Cells	C	BV480	CD161 (REA631)				1	3				[0.5 of 1x]	
11	V7	1.25	Cells	C	BV510 (dim)	CD45RA (HI100)						1.0	3.0			
21	V10	1.25	Cells	C	BV605		CD56					1.25	3.75			
22	V11	1.25	Cells	C	BV650	CCR7 (G043H7)	HLA-DR {0.8}					~~1.0~~ 1.25	~~3.0~~ 3.75			
23	V13	1.25	Cells	C	BV711		CCR7			1.25	3.75					
	V14	1.25	Cells	C	BV750	IFNg (B27)									[1.5 of 1x]	
12	V15	1.25	Cells	C	BV786	CCR6 (11A9)				1.8	5.4					
13	B2	1.25	Cells	C	Alexa488/FITC	hCD1d/Va24Ja18	•		<1:750>			1	3			
14	B3	1.25	Cells	C	Spark blue 550	CD3 (SK7)						1.2	3.6		2.5	7.5
24	B4	1.25	Cells	C	PE	PD-1 (EH12.2H7)	NKG2D			↑		1.25	3.75		2.5	7.5
15	B6	1.25	Cells	C	PE-CF594	CD26 (M-A261)				1.2	3.6					
16	B8	1.25	Cells	C	PE-Cy5	CD25 (M-A251)						1.2	3.6		2.5	7.5
	B10	1.5 (<=10^4)	Cells	C	PerCP-Cy5.5	TNFa (MAB11)									[2.5 of 1x]	
25	B13	0.5	Cells	C	PE-vio770	~~HLA-DR (REA805)~~	PD1					↑ ~~0.5~~	~~1.5~~ 3			
	R1	1.25	Cells	C	APC		IL-4									
17	R2	1.25	Cells	C	Alexa Fluor 647	hMR1			<1:500>						[1.[illegible] of 1x]	
	R4	1.25	Cells	C	APC-R700	CD107a (H4A3)	6.0									
18	R6	1:2500	Cell	P	Zombie NIR	Viability	•	<1:2500>								
19	R7	1.3	Cells	P	APC/Fire 750	CD27 (O323)	•			2	6					
20	R8	1.3	Cells	C	APC/Fire 810	CD38 (HIT2)				1.6	4.8					
						Total volume	6.8			12.1	36.3	16.8	50.4			

HH-cytoFP(2) to match signal at 10^5

unstained Beads

unstained Cells

23 color PBMC

Brillian Staining Buffer

Each reaction 50ul | 50 per rxn ul | 50 per rxn ul | 50 per rxn

Almost missed! Will Fitc CD27 & CD8 ✓ unstained

Protocol

- Aliquot cells
- PMA-Ionomycin stimulation
- PBS 2ml — 1400rpm, 6min
- Live/Dead @RT 15min
- PBS-FBS 2ml — 1400rpm, 6min
- Tetramers @RT 40min
- PBS-FBS 2ml — 1400rpm, 6min
- Surface 37oC 30min: CCR4, CXCR3, CD161, ~~CXCR5~~, CCR6, CD26, CD27, CD38
- PBS-FBS 2ml — 1400rpm, 6min
- Surface mix @ 4oC 30min
- Lysing solution 300-500ul
- PBS-FBS 2ml — 1400rpm, 6min
- Cytofix/perm @ 4oC 20min
- wash twice PermWash 1 ml — 1500rpm, 6min
- Intra Staining @ RT for 40min
- wash once w/ 2ml — 1500rpm, 6min
- Resuspend 100 ul PFA-PBS

sc's
← stim
← wash 200
← RBC lysis
← wash
Fix/Perm
wash/wash
resuspend

CCR5	605/650 HLA-DR 56
Perforin	711 CCR7
GrzmA	PE NKG2D
	PE-Vio PD1
	APC Il-4

Need! - new unstained CBMC
- new CD8 "
- new Fitc! "
- new CD27! "
- new L/D "

Run all in library

- Stim: Run all existing 23 sc's + existing cytokines (PMA)
(↑ thaw extra CBMC tube)

- Adult: run all sc's as phenotype & run all as stim controls (PMA)

Extra PBMCs (if needed) ← CQ 1070 #8611, CQ 1106 #8640

↑ 400ul L/D & unstained

1.6 + .6 = 2.2

1.6 + .8 = 2.4

.2/4.19 = 50µl

.4/4.19 = 100µl

Sept 15. → Failed UV3 6.78 (5.23)

17 7.00

21 6.55

Sept 31st Blue Esc failed (6.89) UV 8.00

Oct 5th → SSC-B failed 8.65 UV 3 8.73

13th → 6.81

% r CV
(<6%)