

October 4-6th, 2021

HEU: Ex-vivo $\gamma\delta$ CBMC #4 - Aurora ILT #1

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	1E+6	1.5E+6
Inf029				2021-07-22							
NDO50				2021-08-24							

Thawed ~ 10:30 AM. Aliquoted ~ 3pm @ 600k cells (10/04/2021), 4%

@ 6:27 Scls addition BUVs start

@ 7:05 H₂O₂ → fridge for 30 min @ 7:35
37°C → incubator

★ New FBS aliquot (PMA side process)

@ 7:44pm → 1ml FBS-FBS wash, spin @ 1300rpm 8 min

@ 20:09pm - FixPerm first vortex
first wash @ 20:23 pm
2nd wash @ 20:37 pm

PFAed (bulk tubes) @ 8:56

Intracellular @ 8:58pm → 9:38pm

Done @ 9:50pm

Tuesday - 1 cord Inf 29

- 1 adult NDO50

Still need to make 1 L/D well
1 CD27 sc

L/D @ 2:10pm

Levamisole @ 38°C stain - 4:00pm

FixPerm @ 5:23pm

Done @ 6:07pm

response @ 1.25 µl

x4 x4

#	Filter	Single color (ul)	Ref ctrl	Fluorochrome	Marker	Drugging stimulation	Live/Dead RT for 15min	RT for 10min	37°C (RT) for 30min	Surface staining: 4°C for 30min	4°C for 15min	Lysing, then CytoFix/Perm	RT for 40min Universal lysis mix (10x diluted)
1	UV2	0.5	Cells	BUV395	CD62L (PREG-56)					1.2 4.8			2.5
2	UV7	0.5	Cells	BUV496	CD8 (PRA-18)					1.0 4			
3	UV9	0.2	Cells	BUV563	CD69 (FEN50)					1.0 4			
4	UV10	0.6	Cells	BUV615	CCR4 (JG1)					1.0 4			
5	UV11	0.5	Cells	BUV661	VD2 (B6)					1.0 4			
6	UV14	0.5	Cells	BUV737	CCR3 (T6/CCR3)					1.0 4			
7	UV16	1.0	Cells	BUV805	CD4 (S3X)				2.5 10	1.5 6			2.5
8	UV1	0.8	Cells	BUV421	CD127 (A01905)					1.0 4			
9	UV3	0.2	Cells	Pacific Blue	CD14 (M5E2)					2.0 8			
10	UV5	0.2	Cells	Pacific Blue	CD19 (H1B19)					2.0 8			
11	UV7	1.0	Cells	BUV480	CD161 (RFA631) (direct)					1.0 4			
12	UV8	1.0	Cells	BUV510 (dim)	CD45RA (H100)					1.0 4			
13	UV10	0.5	Cells	BUV605	CCR5 (J252D4)					1.0 4			
14	UV11	0.2	Cells	BUV650	CCR7 (G043H7)					1.0 4			
15	UV13	0.2	Cells	BUV711	IFNγ (B27)					1.0 4			
16	UV15	0.8	Cells	BUV786	CCR6 (T1A9)					1.8 7.2			
17	UV2	0.2	Cells	Alexa488/ITC	hCD1d/Na24a1a18					1.2 4.8			
18	UV3	0.5	Cells	Spark Blue 550	CD3 (SK7)					1.2 4.8			
19	UV4	0.2	Cells	PE	PD-1 (EH12.2H7)					1.2 4.8			
20	UV6	0.2	Cells	PE-CF594	CD26 (M-A261)					1.2 4.8			
21	UV7	1.5 (x=10x4)	Cells	PE-Cy5	CD25 (M-A251) (P-A251)					1.2 4.8			
22	UV13	0.8	Cells	PE-vio770	HLA-DR (REA805) (M6)					0.5 2			
23	UV1	0.8	Cells	APC	CD39 (A1)					1.2 4.8			
24	UV2	0.2	Cells	Alexa Fluor 647	HMβ1					1.2 4.8			
25	UV3	0.2	Cells	APC-R700	CD107a (H4A3)					1.2 4.8			
26	UV4	1.2500	Cell	Zombie NIR	Viability					1.2 4.8			
27	UV7	1.0	Cells	APC/Fire 250	CD27 (G323) (P-A251)					1.2 4.8			
28	UV8	1.0	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
29	UV10	1.25	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
30	UV16	1.0	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
31	UV1	0.8	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
32	UV2	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
33	UV3	0.5	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
34	UV4	1.0	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
35	UV5	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
36	UV6	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
37	UV7	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
38	UV8	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
39	UV9	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
40	UV10	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
41	UV11	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
42	UV12	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
43	UV13	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
44	UV14	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
45	UV15	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
46	UV16	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
47	UV17	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
48	UV18	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
49	UV19	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
50	UV20	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
51	UV21	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
52	UV22	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
53	UV23	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
54	UV24	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
55	UV25	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
56	UV26	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
57	UV27	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
58	UV28	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
59	UV29	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
60	UV30	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
61	UV31	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
62	UV32	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
63	UV33	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
64	UV34	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
65	UV35	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
66	UV36	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
67	UV37	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
68	UV38	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
69	UV39	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
70	UV40	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
71	UV41	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
72	UV42	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
73	UV43	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
74	UV44	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
75	UV45	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
76	UV46	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
77	UV47	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
78	UV48	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
79	UV49	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
80	UV50	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
81	UV51	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
82	UV52	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
83	UV53	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
84	UV54	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
85	UV55	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
86	UV56	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
87	UV57	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
88	UV58	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
89	UV59	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
90	UV60	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
91	UV61	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
92	UV62	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
93	UV63	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
94	UV64	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
95	UV65	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
96	UV66	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
97	UV67	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
98	UV68	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
99	UV69	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			
100	UV70	0.2	Cells	APC/Fire 810	CD38 (H17)					1.2 4.8			

1:2500
NIR
5Ml
1ml

26 colors

2 1 1 1

$$S = \frac{2}{10} \times 14 = 28$$

Protocol	Aliquot cells	1000rpm, 6min
	see stimulation	1200rpm, 6min
	gold blockers	1200rpm, 6min
	PBS 5ml	1200rpm, 6min
	Live/Dead	1200rpm, 6min
	PBS-FBS 2ml	1200rpm, 6min
	CD161-biotin @ RT 15min	1200rpm, 6min
	CD39	1200rpm, 6min
	CD39	1200rpm, 6min
	CD27	1200rpm, 6min
	CD38	1200rpm, 6min
	PBS-FBS 2ml	1200rpm, 6min
	Surface mix @ 4°C 30min	1200rpm, 6min
	PBS-FBS 2ml	1200rpm, 6min
	STREP-IVB70	1200rpm, 6min
	Lysing solution 300-500ul	1200rpm, 6min
	PBS-FBS 5ml	1200rpm, 6min
	Cytofix/perm @ 4°C 20min	1200rpm, 6min
	Wash twice	1200rpm, 6min
	Intact staining @ RT for 40min	1200rpm, 6min
	Wash once w/ 2ml	1200rpm, 6min
	CD39	1200rpm, 6min
	IL-4	1200rpm, 6min
	CD161	1200rpm, 6min
	CD56	1200rpm, 6min
	PDI	1200rpm, 6min

1600rpm
PFA
P3C

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October 4-6th, 2021

HEU: Ex-vivo $\gamma\delta$ CBMC #4 – Aurora ILT #1[illegible]

III

Teftamer

$A = 647 \text{ m}^2$

AT400 head

1

Unloaded G-FFP

- Filizade

CPMC

Fixed

✓
St melted ✓
✓

6h

PHX-12

CBMC stim

321

22

13



Q

22

4

1950

DATE	DESCRIPTION	AMOUNT
1/1
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1951

DATE	DESCRIPTION	AMOUNT
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1952

DATE	DESCRIPTION	AMOUNT
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1/30

October 4-6th, 2021

HEU: Ex-vivo $\gamma\delta$ CBMC #4 - Aurora ILT #1

Inf 029
Est rate ~ 2500 ° flow rate for $\sim 13 \mu\text{L}/\text{min}$

Run new Ref controls, appended 100,000 cells for VD2 (+15000 original),
everything else run \sim on.

☐ tube

Acquisition

See sasas edit

☐ tube ☐ Group

run initial, run unstained, run remainder
all in the default raw worksheet.

- ref group unstained
- Folder
 - cells #1
 - cells #2

Live UNmixing

CD38 \leftarrow 26 marker
AF647 \rightarrow 5th pop / CD31
CD8 \leftarrow CD4 b.t. below
extra 5th or CD4 \leftarrow CD4
CD26 \times CD22 \rightarrow 2nd pop

1940

1940

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1940

October 4-6th, 2021

HEU: Ex-vivo $\gamma\delta$ CBMC #4 – Aurora ILT #1

Cytemeter 1.5

Fluids shutdown.

Bleach water control bleach

NGT

Avg 180000

[Skip this time]													[Skip this time]	
#	Filter	Single color (u)	Ref ctrl	Fluorochrome	Marker	Dyeing stimulation	Live/Dead RT for 15min	RT for 10min	37°C (RT) for 30min	Surface staining 40°C for 30min	40°C for 15min	Lysing, then CytoFix/Perm	RT for 40min Universal Intra mix (10x diluted)	
1	UV2	0.5	Beads	BUV395	CD62L (PREG-56)					1.2 2.4			2.5	
2	UV7	0.5	Beads	BUV496	CD8 (RPA-18)					1.0 2				
3	UV9	0.2	Beads	BUV568	CD69 (FNSO)					1.0 2				
4	UV10	0.6	Beads	BUV615	CCR4 (IG1)					1.0 2				
5	UV11	0.5	Beads	BUV661	Vα2 (B6)					1.0 2				
6	UV16	1.0	Beads	BUV737	CCR3 (166/CCR3)					1.0 2				
7	V1	0.8	Beads	BUV805	CD4 (S33)				2.5	1.5 3			2.5	
8	V3	0.2	Beads	BUV421	CD127 (A01905)					1.0 2				
9	V3	0.2	Beads	Pacific Blue	CD14 (M5E2)					2 4				
10	V5	0.2	Beads	Pacific Blue	CD19 (H1819)					2 4				
11	V7	1.0	Beads	BUV480	CD133 (RPA63.1) Biotin				1 2	2 4				
12	V10	0.5	Beads	BUV510 (dim)	CD45RA (H1200)					1.0 2			[0.5 of 1x]	
13	V11	0.2	Beads	BUV605	CCR5 (J22204)				2.5	1.0 2				
14	V13	0.2	Beads	BUV650	CCR7 (G043H7)					1.0 2				
15	V14	0.2	Beads	BUV711	Vβ2.2 (EC10)					1.0 2				
16	V15	0.8	Beads	BUV750	IFNγ (B27)					1.0 2			2.5	
17	B2	0.2	Beads	BUV786	CCR6 (11A9)				1.8	1.2 2.4			[1.5 of 1x]	
18	B3	0.5	Beads	Alexa488/ETC	hCD1d/α24A18					1.2 2.4				
19	B4	0.2	Beads	Spark blue 550	CD3 (SK7)					1.2 2.4			2.5	
20	B6	0.2	Beads	PE	PD-1 (E11.2.847)					1.2 2.4			2.5	
21	B8	0.2	Beads	PE-Cy5	CD25 (M-A251)				1.2	1.2 2.4			2.5	
22	B10	1.5 (C-10x4)	Beads	PerCPviolet700	INRA (M-B11)					1.2 2.4			2.5	
23	B13	0.8	Beads	PE-vio770	HLA-DR (F88805)					0.5 1			[2.5 of 1x]	
24	R1	0.8	Beads	APC	CD39 (A1)				1.2	1.2 2.4			[1.2 of 1x]	
25	R2	0.2	Beads	Alexa Fluor 647	IFNγ1					1.2 2.4				
26	R4	0.2	Beads	APC-R700	CD107a (H4A3)					1.2 2.4				
27	R6	12500	Cell	Zombie NIR	Viability					<1.2500%				
28	R7	1.0	Beads	APC/Fire 750	CD27 (G323)				2 4					
29	R8	1.0	Beads	APC/Fire 810	CD38 (H12)				1.6	1.6 3.2				
HH-cytoFp2) to match signal at 10x5														
Total volume 7.6														
0.8														
50 per rxn 60.50 158 42.5 17.6														
50 per rxn 60.50 158 42.5 17.6														
50 per rxn 60.50 158 42.5 17.6														

Tuesday

IL-4/TNF-IL-6

26 colors

50 per rn
60.5ul
61.5

CCR4
CCR3
CCR5

CD26
CD25
CD27

1111

1111

Protocol	
Allyl cells	
SEB stimulation	
Golgi blockers	
PBS 2ml	1400rpm, 6min
Live/Dead	
PBS-FBS 2ml	1400rpm, 6min
CD161-biotin @ RT 15min	
CCR3	CD26
CCR5	CD39
CCR6	CD27
CCR4	CD38
PBS-FBS 2ml	1400rpm, 6min
Surface mix @ 40C 30min	
PBS-FBS 2ml	1400rpm, 6min
Strept BV480	
Lysing solution 300-500ul	
PBS-FBS 2ml	1400rpm, 6min
CytoF/perm @ 40C 20min	
Wash twice	1500rpm, 6min
Intra Staining @ RT for 40min	
Wash Once w/ 2ml	1500rpm, 6min
CCR5	GrnA
IL-4	NG2D
CD107	Perforin
CD56	
PBS	

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100