

October 4-5th, 2021

HEU: Ex-vivo $\gamma\delta$ CBMC panels #3

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	1E+6	1.5E+6
Inf305-8 2-2 (7)	SM ZV HU	Box 9A E.5		2019-09-30	Scs & Gamma G00K	2	10E6	12.5E6	20 M		150 μ l
Inf 188 229-3 (8)	ScFB HEU-L	Box 5 7A B.7, B.8		2019-10-10 2019-04-20		2	4.4E6	6.79E6	8.8 M		334 μ l
Inf314-8 2-2 (9)	SM H.B HEU-H	Box 10A, B.7		2019-11-05	1.5 = 11.2 M	2	7.45E6	9.33E6	14.9 M		201 μ l
ND050	Adult		15E6	08/24/21	.5 = 1.7 M	2	3.51E6	4.31E6	7.02	113 μ l	

	Ex vivo Gd-p4b	Surface μ L x 3	Intra μ L x 3
BV421	PD1	2	6
BV510	Aqua L/D	1:500	
BV650	CD56	1.8	5.4
Alexa 488 (FITC)	Perforin (dG9)	-	3
PerCPeF710	CD3	1.5	4.5
PE	GZMb	-	1.5
PE Dazzle			4.5
PE Vio770	NKG2A	0.6	1.8
APC	V82	1	3
APC Fire750	CD16	1	3
20.5 μ l/rxn;	PBS	12.6	37.8

	Ex vivo Gd-CK PMA	Surface μ L x 6	Intra μ L x 6
BV421	PD1	2	12
BV510	Aqua L/D	1:500	
BV650	CD27	1.5	9
FITC	V82	1.2	7.2
PerCPeF710	CD3	1	6
PE	CD56	0.5	3
PE Dazzle			
PE Vio770	IFN γ	-	0.6
Alexa 647 (APC)	TNF α	-	2
APC Fire750	CD45RO	2	12
20.5 μ l/rxn;	PBS	12.3	73.8

	Ex vivo Gd-p1	Surface μ L x 3
V450 BV421	PD1	2
V525 BV510	Aqua L/D	1:500
V670 BV650	CD16	1.5
B530 FITC	V82	1.2
B710	CD25	2
PerCPeF710	CD28	2
Y590 PE	CD27	1.5
Y615 PE Dazzle	CD3	0.5
Y780 PE	CD3	0.5
Vio770	CD3	0.5
R670 APC	V81	1
R780	CD45RA	1.8
APC Fire750	CD45RA	1.8
20.5 μ l/rxn;	PBS	7

	Ex vivo Gd-p2	Surface μ L x 3	Intra μ L x 3
BV421	V82	1	3
BV510	CD3	1.5	4.5
BV650	NKG2D	2	6
	Streptavidin	{1.5}	
Alexa 488 (FITC)	dg9	-	3
PerCPVio700	CD56	1	3
PE	PD1	1.5	4.5
PE Dazzle			
PE Vio770	NKG2A	0.6	1.8
APC	V81	1	3
APC Fire750	Horizon L/D	1:1000	
20.5 μ l/rxn;	PBS	11.9	35.7

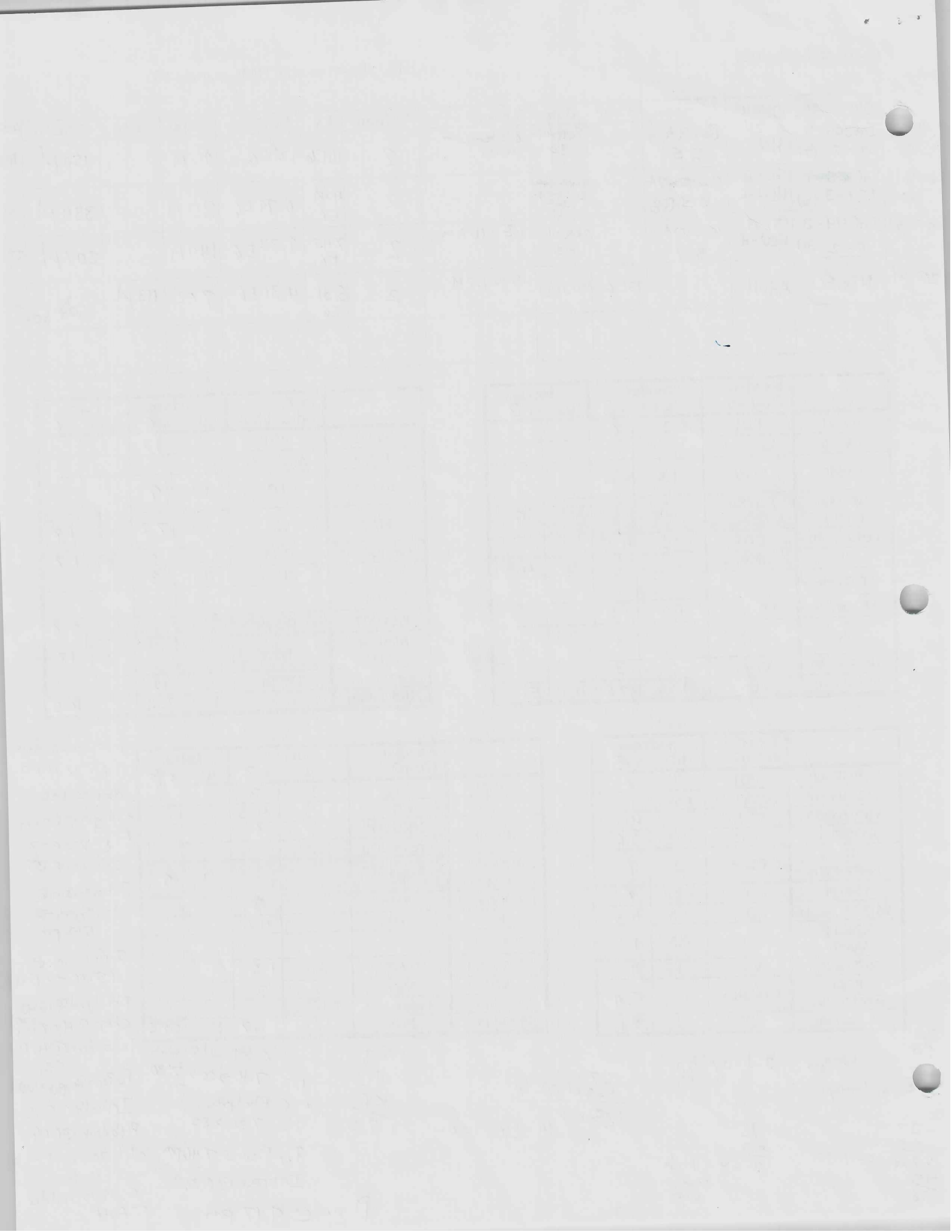
10:50 count stain
PMA @ 12:26 p.m.
APC @ 1:22
First spin @ 2:03 pm
Wash @ 2:26
Scs in @ 2:35
Tetraspan @ 15:15 pm
15:55 pm
Surface in @ 15:21
Rbc lysis @ 15:48
Strep @ 4:07 pm
Tetraspan @ 4:11
FixPerm @ 5:15
P4b down @ 6:04
ctrl 3057 - 8 ml
L/D Aqua
G:39 L/D added
C:6:46

3
24
3
12.2 20pl - 40pl
27
3
87 ml FB/PBS
115
96
2 tubes

3 ml - 3 pl 1/2
Wash on
12
3
36 - 4 - 1 tube
Perm
Wash

27
x 500pl RBC lysis
13500
1A - 1.4 = 12.6 ml

53
19.5
6
7.0
6:58 2ml FB wash
7:10 - 20 - 7:30
x 6 Rbc lysis
7:32 - 35
FixPerm @ 7:46 pm
Intra @ 8:26
Done @ 9:17 pm



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890 c/p Inf305-8 a-2	SM20 HU				400 pl	2	10 E6	12.5 E6	20 M		1.5 E+6
600 c/p Inf229-3 a-2	SCFA HEU-L				x	2	4.17 E6	6.73 E6	8.34 M	1.5 .360 pl	2400 pl + 760
890 c/p Inf314 a-4	SWHA HEU-H				58 pl	2	6.92 E6	8.94 E6	13.8 M		4500 pl + 550
							3.51 E6				3400 pl + 600
											350 pl + 600

$$\begin{aligned}
 & \text{a1} \quad \sim \\
 & 17.4 + 20 = 37.4 - 13.5 = 23.9 \\
 & 6.46 + 8.8 = 15.26 - 13.5 = 1.76 \\
 & 11.49 + 14.9 = 26.39 - 13.5 = 12.89 \\
 & 5.79 = 400 \text{ pl after sc's} \rightarrow 1 \text{ tube}
 \end{aligned}$$

$$\begin{array}{r}
 .4 \\
 \times 30 \\
 \hline
 12.0
 \end{array}$$

030 4026

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	Ex vivo MAIT-p1a	Surface 3 μL x 4.5	
V450 BV421	Va7.2	2	6
V525 BV510	Aqua L/D	1:500	-
V670 BV650			
B530 FITC	CD4	1.8	5.4
B710			
PerCPeF710	CD45RA	2	6
Y590 PE	CD161	0.5	1.5
Y615 PE Dazzle	CCR7	1.5	4.5
Y780 PE			
Vio770	CD3	0.5	1.5
R670 APC	CCR6	2.5	7.5
R780			
APCFire750	CD8	1.5	4.5
20.5 $\mu\text{L}/\text{rxn}$;	PBS	8.2	24.6

760 μL In 229 ml
2180 μL In 229 ml

	Ex vivo MAIT-p1a	Surface 3 μL x 4.5	
V450 BV421	CCR6	2	6.5
V525 BV510	Aqua L/D	1:500	-
V670 BV650			
B530 FITC	CD4	1.8	5.4
B710			
PerCPeF710	CD45RA	2	6
Y590 PE	CD161	0.5	1.5
Y615 PE Dazzle	CCR7	1.5	4.5
Y780 PE			
Vio770	CD3	0.5	1.5
R670 APC	hMR1	(4.6) 5 μL	-
R780			
APCFire750	CD8	1.5	4.5
20.5 $\mu\text{L}/\text{rxn}$;	PBS	10.7	32.1

	Ex vivo NKT-p1a	Surface 3 μL x 4.5	
V450 BV421	Va24	1.2	3.6
V525 BV510	Aqua L/D	1:500	-
V670 BV650	NKG2D	1	3
B530 FITC	CD4	1.8	5.4
B710			
PerCPeF710	CD45RA	2	6
Y590 PE	CD161	0.5	1.5
Y615 PE Dazzle	CCR7	1.5	4.5
Y780 PE			
Vio770	CD3	0.5	1.5
R670 APC	CCR6	2.5	7.5
R780			
APCFire750	CD8	1.5	4.5
20.5 $\mu\text{L}/\text{rxn}$;	PBS	8	24

18.5 draws

	Ex vivo NKT-p1a	Surface 3 μL x 4.5	
V450 BV421	CCR6	2	6.5
V525 BV510	Aqua L/D	1:500	-
V670 BV650	NKG2D	1	3
B530 FITC	CD4	1.8	5.4
B710			
PerCPeF710	CD45RA	2	6
Y590 PE	CD161	0.5	1.5
Y615 PE Dazzle	CCR7	1.5	4.5
Y780 PE			
Vio770	CD3	0.5	1.5
R670 APC	hCD1d	1:10 (1.5)	-
R780			
APCFire750	CD8	1.5	4.5
20.5 $\mu\text{L}/\text{rxn}$;	PBS	9.7	29.1

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

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