

August 17th, 2023

2023_Conventionals_01

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
Inf105-6 a-3	HU ♂ RYK8	(5)	47.217			1	12.4*	19.8	80/120
Inf185-4 a-6	HU ♀ S941	(6)	24.37			1	4.07*	6.5	245/368
Inf064-0 a-3	HEU-10 RW7N	(3)			red cap LNZ inside	1	4.94*	8.06	202/303
Inf173-1 a-3	HEU-10 ♀ S98 N	(7)	416.27			1	4.02*	7.7	248/373
Inf034-2 a-8	HEU-10 RUT9	(4)	4397		orange cap LNZ inside large clump	1	14.93	2.4	416/625
Inf052-7 a-4	HEU-10 ♀ RUJD	(2)	44.47		normal viral	1	13.6*	19.9	74/110
ND050	Adult ♂ ctrl	(1)	7.667	6/5/23		1	12.8	16.9	78/117

< 3hr prep work >

Start @ 12:15 pm 12:32 spin

Stain for count @ 1:44 pm

Count on NFR-B log due to no PE CD45

2:37 Intrac for Inf105 & Inf053

Cells spin @ 2:58 pm

3:58 pm L/D for conc.

Spin @ 4:17 pm

4:22 pm sc's for 20 min 4:41 spin

Surface @ 4:33 pm → 4:53 pm

Spin @ 4:54 (no direct lyse)

5:06 RBC lyse spin @ 5:13

5:26 strept → spin @ 5:43 pm

Pi w/ 30 pl PFA-PBS

Fix Perm @ 5:58 pm

< cyt mix made @ 6:07 >

1st Perm Wash spin @ 6:23 pm

end @ 6:35

Intrac @ 6:48 pm (sc's w/ 50 pl)

Final spin @ 7:28 pm

Done @ 7:41 pm

For HIV testing:

1 Million / 1 ml w/ 5 pl (10 ug) IL-2
w/ 2 pl PHA-PC (Yutaka)
@ 10 →

Step frozen aliquots, spin, moved, added 50
pl, remove 500g for 7 min, removed sup
& freeze in Dewar

Spin cells @ 8:42 pm

8:51 pm L/D

9:06 pm spin

9:17 pm → 37 pm surface

9:44 pm RBC lyse spin

Fix Perm @ 9:54 → 04 → H

10:17 first wash

10:23 second

10:34 start → 11:14 out

Final wash @ 11:15 pm

Done @ 11:24 pm

collection @ 12:31 pm

P4b 3000 cuts;

Info 64 had decent Vd2's

~5 minutes/each
(still lot residual
volume @ med)

//Info 39 → mostly dead//

Stop recording → recording → append

PT

BT

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Ex-vivo Gd-P4b									
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	6	Intracellular 40min @RT	6
1	V450	BV421	PD1			2	12		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD56			1.8	10.8		
4	B530	Alexa 488	Perforin					3	18
5	B710	PerCPeF710	CD3			1.5	9		
6	Y590	PE	GzmB					1.5	9
7	Y780	PE-Vio770	NKG2A			0.6	3.6		
8	R670	APC	Vδ2			1	6		
9	R780	APC-Fire750	CD16			1	6		
Antibody Total						7.9	47.4	4.5	27
PBS						12.6	75.6	16	96
Pipette draw volume / sample						19.5		19.5	

Ex-vivo Gd-P2										
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	6	Streptavidin	Intracellular 40min @RT	6
1	V450	BV421	Vδ2			1	6			
2	V525	BV510	CD3			1.5	9			
3	V670	BV650	NKG2D			2	12	1.5		
4	B530	Alexa 488	Perforin							
5	B710	PerCPeF710	CD56			1	6		3	18
6	Y590	PE	PD1			1.5	9			
7	Y780	PE-Vio770	NKG2A			0.6	3.6			
8	R670	APC	Vδ1			1	6			
9	R780	APC-Fire750	L/D Horizon		<1:1000>					
Antibody Total						8.6	51.6	9	3	18
PBS						11.9	71.4	114	17.5	105
Pipette draw volume / sample						19.5		19.5	19.5	

Ex-vivo Gd-P1									
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	6		
1	V450	BV421	PD1			2	12		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD16			1.5	9		
4	B530	FITC	Vδ2			1.2	7.2		
5	B710	PerCPeF710	CD25			2.5	15		
6	Y590	PE	CD28			2	12		
7	Y615	PE-Dazzle	CD27			1.5	9		
8	Y780	PE-Vio770	CD3			0.5	3		
9	R670	APC	Vδ1			1	6		
10	R780	APC-Fire750	CD45RA			1.8	10.8		
Antibody Total						14	84		
PBS						6.5	39		
Pipette draw volume / sample						19.5			

Ex-vivo gd-CK: PMA-ionomycin									
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	12	Intracellular 40min @RT	12
1	V450	BV421	PD1			2	24		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD27			1.5	18		
4	B530	FITC	Vδ2			1.2	14.4		
5	B710	PerCPeF710	CD3			1	12		
6	Y590	PE	CD56			0.5	6		
7	Y780	PE-Vio770	IFN γ					0.6	7.2
8	R670	Alexa 647	TNF α					2	24
9	R780	APC-Fire750	CD45RO			2	24		
Antibody Total						8.2	98.4	2.6	31.2
PBS						12.3	147.6	17.9	214.8
Pipette draw volume / sample						19.5		19.5	

γδ T cell p1										8/17/2023		
Spectrum		Violet		Blue				Red				
428	V1	BV421	[4]	PD1	B2	FITC	[1.5]	Vδ2	APC Alexa 647	[3.5]	Vδ1	
525	V6	BV510	[1.5]	L/D Aqua	B3		PE	[4]				CD28
542	V7				B4			[4]				CD27
582	V8				B5							
598	V9	BV650	[3.5]	CD16	B6	PE-Dazzle594	[4]	CD27	APC-Fire 750	[2]	CD45RA	
613	V10				B7		[3]	CD25 CD3				
664	V11				B8							
679	V13				B10							
717					B13							
783					B14							
812		V16										

$\gamma\delta$ T cell p2								
Spectrum	Violet		Blue		Red			
428	V1	BV421	[4]	V δ 2				
525	V6			Alexa 488	[1.5]	Perforin		
542	V7	BV510	[1.5]	CD3				
582	V8			PE	[4]	PD1		
598	V9							
613	V10			PE-Dazzle594	[4]			
664	V11	BV650	[3.5]	NKG2D			R1 APC R2 Alexa 647	[3.5] V δ 1
679							R4	
717	V13			PerCP-Vio700		CD56	R7	
783	V15			Pe-Vio770	[3]	NKG2A	R8	L/D Horizon
812	V16							

[illegible][illegible]

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