

August 27, 2023

2023\_Conventionals\_02

15E16

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
1270c. Inf185-4 30pl a-7	HU ♀ 5982	5B, F9 (2)	~6.5		<u>cl only</u> clean LAM clump	0.75	8.72	12.7	6.54/120pl
1100c. Inf694-9 30pl a-1	HU ♀ 7NRY	(7)	~8.5 <7.93>		Set chunk dying	0.75	12.6	18.2	4.45/120pl
1500c. Inf809-7 30pl a-3	HU ♂ U6x8	(8)	~12 <10.7>		Set minor m clump	0.75	10.8	13.6	8.1/140pl
1460c. Inf064-8 30pl a-6	HEU-b ♂ RW7R	(10)	~6 <13.8>		<u>cl only</u>	0.75	7.64	12.0	5.73/200pl
1190c. Inf100-1 30pl a-7	HEU-b ♂ RY9N	(5)	~4.5 <20.9>		Set dying mon	0.75	4.43	10.4	6.24/360pl
1280c. Inf100-1 30pl a-8	RY9P	(1)	~23.5		dying mon	0.75	3.89	8.53	
1380c. Inf173-1 30pl a-5	HEU-b ♀ 598Q	(4)	~5.5 <12.5>		<u>cl only</u>	0.75	5.09	10.7	3.92/200pl
1370c. Inf479-0 30pl a-3	HEU-b ♀ T32N	(17)	~9 <6.4>		Set clump	0.75	12.30	18.5	9.23/120pl
1290c. Inf320-5 30pl a-4	HEU-b ♀ JNV1	(11)	~7.5 <11.6>		Set minor drops minor mon	0.75	7.06	8.55	5.3/210pl
170c. Inf342-9 30pl a-4	HEU-b ♀ SQ3R	(6)	~4.0		Set low signal? minor mon	0.5			
125c. Inf342-9 30pl a-3	SQ3Q	(9)	~5.11		minor mon	0.5	7.97	12.5	7.47/120pl
140c. Inf387-8 30pl a-7	HEU-b ♀ SVWG	(3)	~4.5 <22.8>		Set energy m	0.75	4.61	6.54	
1350c. Inf387-8 30pl a-6	SVWS	(12)	~31.2		some dy m	0.75	3.58	5.48	6.14/370pl

Reagents & Abs prepped @ 3:51 pm

Thaw 1 4:41 pm spin 4:55 5:11 DNAse

gt ~~Antico~~ 5:20 spin 5:29 DNAse

6:13 pm stain for count 5:46 DNAse

7:26 extras bridge

7:43 pm Incubation start

→ 1:43 am

8:36 pm spin of others

[809 + 100-1 P2 inadvertently combined together !!]

Leftovers spinning for snap freeze @ 8:43 pm

supernatant removed & pellet resusp @ 9:00 pm

9:08 pm L/D start → 23

single colors @ 21:13 → 33

9:41 pm ~~Surface~~ → 10:01 am

⊗ All P1 + P2, 3 spec w P4b  
reason being PDI/VD2 in P2 on Δ channel  
~ better stain, NRG20 × NRG2A → VD1,  
P1 covers col6, greenBis meh anyways.

⊗ Single colors started

P1st spins @ 9:58 pm

→ spin (✓) RBC lysis !!

PHA's stacked sup dumped @ 10:12 pm

< 10:17 pm RBC lysis >

10:32 pm all abs prepped

10:36 pm strep → 10:51

Fix Prim @ 11:00 pm → 16: → 22

Spin of microcentrifuge tubes @ 11:21 pm

1st spin @ 11:33 pm 11:50 spin

7 ml  
7/1  
L/D  
Aqua  
Horizon

14  
20  
34  
x .8  
27.2 mls

→ 54.4/L L/D  
Aqua

$$\begin{array}{r} 34 \\ \times 4 \\ \hline 136 \end{array} + \begin{array}{r} 7 \\ \times 6 \\ \hline 42 \end{array} = 178 \text{ ml}$$

FBS-PBS

x .9  
= 160

34  
x 4  
136 mls of Permabond

22  
158 mls  
x .9  
= 142

52  
x .3  
15.6 mls  
RBE lysis

17  
15.3  
1.7 RBE lysis

11:58 pm Intra P4b/P2 → 00:38 am

SLs @ 00:02 w/ 30 μl PFA-PBS

Final spin @ 12:43 am

Stashed w/ 20 μl @ 12:57, organized

~~1:43~~ 1:37 am spin down

1:49 am L/D →

2:22 → 2:42 am

RBE lysis @ 2:44 → spin @ 2:51 am

304 Fix Perm → 14 → 24

3:25 First wash

2nd @ 3:37

Intra @ 3:49 am → 4:29 am

Final spin @ 4:27 pm

Done @ 4:41 pm

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LSR seems clean but dropping...  
5:24 pm start

[R] @ 3000 cuts medium speed  
@ 8000 cuts high

+/- Done @ 6:05  
(not stable count @ High)

[P2] @ 6:08 pm

Grabbing coffee, will be right back ← David,

[CH] resumed @ 7:26 after clean

step IFM V525  
viral load  
core

viral load  
@ delv

← for Friday

OT-24

blue med-ol

-24



2/5



August 23rd, 2023

Ex-vivo Gd-P4b									
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	7	Intracellular 40min @RT	7
1	V450	BV421	PD1			2	14		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD56			1.8	12.6		
4	B530	Alexa 488	Perforin					3	21
5	B710	PerCPeF710	CD3			1.5	10.5		
6	Y590	PE	GzmB					1.5	10.5
7	Y780	PE-Vio770	NKG2A			0.6	4.2		
8	R670	APC	Vδ2			1	7		
9	R780	APC-Fire750	CD16			1	7		
Antibody Total						7.9	55.3	4.5	31.5
PBS						12.6	88.2	16	112
Pipette draw volume / sample						19.5		19.5	

\* New NKG2D  
protein

\* NKG2A from  
DO, full version

Ex-vivo Gd-P2										
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	7	Streptavidin	Intracellular 40min @RT	7
1	V450	BV421	Vδ2			1	7			
2	V525	BV510	CD3			1.5	10.5			
3	V670	BV650	NKG2D			2	14	1.5		
4	B530	Alexa 488	Perforin						3	21
5	B710	PerCPeF710	CD56			1	7			
6	Y590	PE	PD1			1.5	10.5			
7	Y780	PE-Vio770	NKG2A			0.6	4.2			
8	R670	APC	Vδ1			1	7			
9	R780	APC-Fire750	L/D Horizon		<1:1000>					
Antibody Total						8.6	60.2	10.5	3	21
PBS						11.9	83.3	133	17.5	122.5
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	7		
1	V450	BV421	PD1			2	14		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD16			1.5	10.5		
4	B530	FITC	Vδ2			1.2	8.4		
5	B710	PerCPeF710	CD25			2.5	17.5		
6	Y590	PE	CD28			2	14		
7	Y615	PE-Dazzle	CD27			1.5	10.5		
8	Y780	PE-Vio770	CD3			0.5	3.5		
9	R670	APC	Vδ1			1	7		
10	R780	APC-Fire750	CD45RA			1.8	12.6		
Antibody Total						14	98		
PBS						6.5	45.5		
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	20	Intracellular 40min @RT	20
1	V450	BV421	PD1			2	40		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD27			1.5	30		
4	B530	FITC	Vδ2			1.2	24		
5	B710	PerCPeF710	CD3			1	20		
6	Y590	PE	CD56			0.5	10		
7	Y780	PE-Vio770	IFN $\gamma$					0.6	12
8	R670	Alexa 647	TNF $\alpha$					2	40
9	R780	APC-Fire750	CD45RO			2	40		
Antibody Total						8.2	164	2.6	52
PBS						12.3	246	17.9	358
Pipette draw volume / sample						19.5		19.5	



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