

February 16th, 2023

HEU: D17 γδ CBMC panel #2 - 2023

Specimen	Status	Location	Conc	Date	Volume	Ly 1:50	Ly+M	Total	1E+6	.66E+6	.5E+6
N4058			31 μl		2 1/2	3.31 / 1.65			606 μl	400 μl	303 μl
Inf 201	hev-6 IL-2		21.7 μl		5 1/2	4.72 / 2.36			423 μl	279 μl	211 μl
	Zol		30 μl		6 1/2	3.43 / 1.71			584 μl	385 μl	292 μl
	BCG		31 μl		6	3.27 / 1.63			613 μl	404 μl	306 μl
Inf 314 (repeat)	HEV+ IL-2		20.5 μl		6	4.94 / 2.47			404 μl	267 μl	202 μl
	Zol		3		6 1/2	4.49 / 2.24			446 μl	295 μl	223 μl
	BCG		22 μl		5 1/2	4.58 / 2.74			364 μl	240 μl	182 μl
Inf 185	IL-2 40 (dead?)	30 sec	Dead								
	Zol		Dead								
	BCG		Dead								

3:45 AM: Guava On

04:06 AM: Cells out incubator

04:28 am: collected

05:01 Guava starts behaving again

< 05:33 am: Cells back incubator >

06:11 am: plate spin!

06:14 am: Plate in, out @ 12:14 pm 11

7:51 am: Reagents prepped >

8:30 am: cells spinning

W/O @ 8:51 am

Cells frozen @ 8:58 pm (6 tubes, 8-12 m)

9:05 sec's → 9:25 pm (spin @ 9:31 am)

9:12 pm spin 1/2

9:27 surface → 9:47 pm

10:04 am streptavidin P₂ spin @ 5:50 pm

→ 10:19 am spin @ 10:22 am

10:33 am → 43 → 53 "

1st perm spin @ 10:55 am

2nd @ 11:05 pm

11:14 am Intracellular → 11:54 am

12:07 pm: Done P₂/P₄ ✓

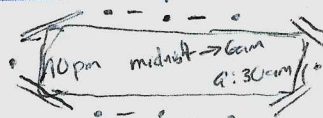
Friday
(tomorrow prep 2000 rpm / 2 min)
Day 14 for Sunday ✓

Friday

NP 0 23 IL-2, BCG, Zol

Inf 136

Inf 587



12:27 pm ck cells spin

W/O @ 12:37 pm → 52 pm
Spin @ 55 pm

surface @ 1:07 pm → 1:27 pm
→ wash @ 1:31

< Perm Wash 1ml, 2 ml FBS > 1400 rpm / 6 min

Fix Perm @ 1:52 pm → 2:02 ✓ → 2:12

Perm Wash #1 / 2nd @ 2:23 pm ✓

Intra @ 2:31 pm → 3:11 pm
Done @ 3:21 pm

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D17 Gd-P4b									
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	9.5	Intracellular 40min @RT	9.5
1	V450	BV421	PD1*			2	19		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD56*			1.8	17.1		
4	B530	Alexa 488	Perforin					3	28.5
5	B710	PerCPeF710	CD3			1.5	14.25		
6									
7	Y780	PE-Vio770	NKG2A			0.6	5.7		
8	R670	APC	Vδ2			1	9.5		
9	R780	APC-Fire750	CD16			1	9.5		
Antibody Total						7.9	75.05	3	28.5
PBS						12.6	119.7	17.5	166.25
Pipette draw volume / sample						19.5		19.5	

D17 Gd-P2										
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	9.5	Streptavidin	Intracellular 40min @RT	9.5
1	V450	BV421	Vδ2			1	9.5			
2	V525	BV510	CD3			1.5	14.25			
3	V670	BV650	NKG2D			2	19	1.5		
4	B530	Alexa 488	Perforin						3	28.5
5	B710	PerCPVio700	CD56			1	9.5			
6	Y590	PE	PD1			1.5	14.25			
7	Y780	PE-Vio770	NKG2A			0.6	5.7			
8	R670	APC	Vδ1			1	9.5			
9	R780	APC-Fire750	L/D Horizon		<1:1000>					
Antibody Total						8.6	81.7	14.25	3	28.5
PBS						11.9	113.05	180.5	17.5	166.25
Pipette draw volume / sample						19.5		19.5	19.5	

D17 Gd-P1							
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	9.5
1	V450	BV421	PD1			2	19
2	V525	BV510	L/D Aqua		<1:500>		
3	V670	BV650	CD16			1.5	14.25
4	B530	FITC	Vδ2			1.2	11.4
5	B710	PerCPeF710	CD25			2	19
6	Y590	PE	CD28			2	19
7	Y615	PE-Dazzle	CD27			1.5	14.25
8	Y780	PE-Vio770	CD3			0.5	4.75
9	R670	APC	Vδ1			1	9.5
10	R780	APC-Fire750	CD45RA			1.8	17.1
Antibody Total						13.5	128.25
PBS						7	66.5
Pipette draw volume / sample						19.5	

D17 gd-CK: α-γδTCR									
#	Detector	Fluorochrome	Marker	Clone	L/D 15 min (RT)	Surface 20 min @4C	9.5	Intracellular 40min @RT	9.5
1	V450	BV421	PD1			2	19		
2	V525	BV510	L/D Aqua		<1:500>				
3	V670	BV650	CD56			1.5	14.25		
4	B530	Alexa 488	CD107a					<3 uL>	
5	B710	PerCPeF710	TNFα					2	14
6	Y590	PE	Vδ2			1	9.5		
7	Y780	PE-Vio770	IFNγ					0.6	5.7
8	R670	Alexa 647	CD27			1.5	14.3		
9	R780	APC-Fire750	CD45RO			1.8	17.1		
Antibody Total						7.8	74.1	2.6	24.7
PBS						12.7	120.65	17.9	170.05
Pipette draw volume / sample						19.5		19.5	

February 15th, 2023

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9:19 am (LSR on) (waste changed)

R670 & V670 ↓ guinea issue
plenty, CD3 staining).

↘ (all other samples have

- Turned off for a while, prepped new sc's, turned on & cleaning.

Contrad @ 9:24 am



Running @ 9:52 ^{sc start} (9:45 AM)

635	FSC		
290	SSC		
R670	510	525	
R780	520	530	
B530	480		
B710	500	540	
V4510	470	490	
V525	550		
V670	480	490	
Y590	595		
Y615	630		
Y780	480		

CU → ~~690~~ FSC.

(710 for dot P2 s. 7)

Erio left afk: ND023 Inf 587, 136.
cells

Running cells @ my concentration 10:57 am

$$\begin{array}{r} 24 \\ \times 3.5 \\ \hline 120 \\ 720 \\ \hline 84.0 \end{array} \quad \begin{array}{c} 12:29 \\ \\ \\ \boxed{1 \frac{1}{2}} \end{array}$$

Stopping @ 12:30 am //

P1 & CU done.

Will keep fingers crossed
& an P4b/P2 tomorrow.

[218 pm machine on]

sc's acquired @ 2:37 pm

↑ ramped up FSC for P4b to 730 //

up to 3 min 30 sec

Bcb, restroom @ 3:34 pm →

acquiring cells cell clot came loose?

P2 @ 3:43 pm

Done @ 4:27 pm

Reinforcing image
as ~~loose~~ states/biography
data scientist/
microbiologist