

October 15-16<sup>th</sup>, 2021

Kirsten eURBPAC  
KATPM

HEU: D17 γδ CBMC panels - #1

after rodent  
(leftover)

Specimen	Status	Location	Conc	Date	Tasks	Volume	Lymphocytes	Total	1E+6	.5E+6	
225 c/p 260 c/p 250 c/p	Inf148	IL-2			12 =	4.8	2.49 EG		402 p1		0.66
		201			12 =	5	2.39 EG			209 p1	0.265
		BC6			14 =	5.4	2.61 EG			191 p1	276
260 c/p 260 c/p 250 c/p	Inf134	IL-2			9.6 =	4.5	2.14 EG		467 p1		252
		201			11.7 =	5.5	2.14 EG			233 p1	308
		BC6			8 =	5	1.61 EG			310 p1	410
130 c/p 150 c/p 240 c/p 190 c/p	Inf159	IL-2			10.75 =	5	2.15 EG		465 p1		307
		201			15.5 =	5.6	2.76 EG			181 p1	239
		BC6			14.4 =	5.5	2.61 EG			192 p1	253
		201			5.1 =	1.4	3.66 EG		109 p1/400k		

	Expanded Gd-p4b	Surface μL x 9.5	Intra μL x 9.5
BV421	PD1*	2	19
BV510	Aqua L/D	1:500	
BV650	CD56*	1.8	17.1
Alexa 488 (FITC)	Perforin (dG9)	-	-
PerCPeF710	CD3	1.5	14.3
PE	CD28	-	-
PE Dazzle			
PE Vio770	NKG2A	0.6	5.7
APC	Vδ2	1	9.5
APC Fire750	CD16	1	9.5
20.5 μl/rxn;	PBS	12.6	119.7

	Expanded Gd-CK PMA	Surface μL x 9.5	Intra μL x 9.5
BV421	PD1	2	19
BV510	Aqua L/D	1:500	
BV650	CD56	1.5	14.3
FITC	CD107a	<3 μL added separately>	
PerCPeF710	TNFα		2
PE	Vδ2	1	9.5
PE Dazzle			
PE Vio770	IFNγ	-	-
Alexa 647 (APC)	CD27	1.5	14.3
APC Fire750	CD45RO	1.8	17.1
20.5 μl/rxn;	PBS	12.7	120.7

	Expanded Gd-p1	Surface μL x 9.5
V450 BV421	PD1	2
V525 BV510	Aqua L/D	1:500
V670 BV650	CD16	1.5
B530 FITC	Vδ2	1.2
B710	CD25	2
PerCPeF710	CD28	2
Y590 PE	CD27	1.5
Y615 PE Dazzle	CD3	0.5
Y780 PE Vio770	CD3	0.5
R670 APC	Vδ1	1
R780	CD45RA	1.8
APC Fire750	CD45RA	1.8
20.5 μl/rxn;	PBS	7

	Expanded Gd-p2	Surface μL x 9.5	Intra μL x 9.5
BV421	VD2	1	9.5
BV510	CD3	1.5	14.3
BV650	NKG2D	2	19
Alexa 488 (FITC)	Streptavidin	{1.5}	
PerCPeF710	dg9	-	-
PE	CD56	1	9.5
PE Dazzle	PD1	1.5	14.3
PE Vio770	PD1	1.5	14.3
APC	NKG2A	0.6	5.7
APC Fire750	VD1	1	9.5
20.5 μl/rxn;	Horizon L/D	1:1000	
	PBS	11.9	113

Used 1.5 pl for my sc's today

12:00 Plate

abs 12:57 pm

Surface stain @ 1:14 pm

13:28 → 500s in

→ 200s 13:35

[180-5] 1:50 stop in

→ 2:05

Stop wash @ 2:01 pm

2 ml FBS

Cyflor @ 2:23 pm

p2/p4b

3:14 pm → Intra

Cells from @

4:07 pm

Preb/p2 done @

4:14 pm

Done @ 9:34 pm ✓

Collected spn @ 6:24

4/0 @ 6:41 → 6:56

Surface @ 7:13 pm

Fix Perm @ 7:52 pm

Intra @ 8:40 pm

5  
27  
x.8  
21.6  
22.5 → 45 p1 L/D  
Aqua

9 ml PBS +  
L/D Horizon  
+ 9 μl



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HEU: D17  $\gamma\delta$  CBMC panels - #1

250  $\mu$ l  
\* 10 tubes

1:48 pm  $\rightarrow$  setup & ready to start:

with D17  
with CBMCs

Perform squashed  $\rightarrow$  500  $\downarrow$   
PDI same  $\rightarrow$  470  $\downarrow$   
BV650  $\rightarrow$  a bit  $\downarrow$  460 (R670 over into)

30mls  
 $\times 2$   
 $\underline{0.60} + 2.4 \text{ ml}$

V450 putting up post sc mark, bit off on chart.  
525 also high

PE pretty high up. (> than P610770)

2:09 recording  $\checkmark$

V670 so much lower control vs real life  
(ass. webster?)

loaded okay on my CBMCs... so shouldn't (?) affect.

Little debris carryover today...  $\checkmark$

FSC  $\rightarrow$  630  $\uparrow$  640 201 Int48 ~ 3min to read

(the PMT seems to be doing ok for p610)

Wash  $\rightarrow$  it's the backflow from the closing of the stage arm....

Intra panel 2 minute collections <sup>PBS</sup>

"Intra sample dilution tube"  
0.8 eth/sec

P2  $\rightarrow$  630 FSC @ 3:04 pm

\* Cnot sheathes up itself, it's the backflush/drop @ end & sheath change  
(appendix)  $\Delta$  pressure

OK  $\uparrow$  640 FSC  $\neq$  630?

IL-2 reflective w/o polarizing slits across specimens.

P1  $\uparrow$  FSC up to 650 for IL-2 ...  
640 for 201

201 nonspecific  
P1 dead stream parallel VD2

Done @ 5:15 pm  $\checkmark$  (3:30)

Perform AF428  
CD3 APC 148 2d

PDI B0421 1c  
CD3 Fite  
CD56 BV650 148 2d  
CD3 Fite

2 Mixer @ DMSO stage  
Infl59  
BC6  
Ted  
201  $\uparrow$  N455  
201  $\uparrow$  N455  
NY055  
BC6

? maybe? unclear...



1584pl  
+ 16pl

7. \$60.00