

November 19-20, 2020

HEU: GD Experiment #2

②

Specimen	Status	Location	Date	Tasks	Volume	Ly	Ly+Mon	Total	1E+6	2E+6
ND006 -512 c/p1	Adult 15E6		3/13/20		2ml	7.61E4	9.11E4	15.22	177μl	52μl sc.
INF 182 2200 c/p1	HU	B6AF5 59.52 a-4	2/5/19		2ml	7.94E4	1.05E5	15.88	188μl	377μl
INF 188 830 c/p1	HEU-L	B6A17 SA3Q a-2	3/13/19		5ml	4.53E4	5.52E4	22.65	331μl	662μl
INF 39 340 c/p1	HEU-H	B2AC1 RUE a-4	8/13/18		3ml	3.79E4	5.09E4	11.37	294μl	74.4μl

8μl + 2ml media

Ex vivo Gd-p4b X 4.5					Ex vivo Gd-CK PMA 6hr X 8.5				
BV421	PD1	2.0	4		BV421	PD1	2uL	17	-
BV510	Aqua	1:500	-		BV510	Aqua LD	1:500	-	-
BV650	CD26	1.0	4.5		BV650	CD27	1.5uL	12.8	
	Strep-bv650	1.5	6.8	in 12+4.5 81μl					
FITC	Perforin (dG9)			3 13.5	FITC	Vd2	1.2uL	10.2	0.2 1.7
PerCPeF710	CD3	1.5	6.8		PerCPeF710	CD3	1uL	8.5	0.2 1.7
PE	GZMb			2 9.0	PE	CD56	0.5uL	4.3	
PE/Dazzle™					PE/Dazzle™				
PE Vio770	NKG2A	0.6	2.7		PE Vio770	IFNg	-	0.6	5.1
APC	CD56	2.0	9.0		APC	TNFA	-	2uL	17
APC Fire750	Vd2	1.8	8.1		APC Fire750	CD45RA	1.8uL	15.3	
20ul/rxn;		10.1	45.5	15.5 69.8	20ul/rxn;		12.5	12.3	17.5 48.8

Ab mix done @ 13:24

cant me @ 2:44pm

Fl: P-Dazzle CD3 vs CD45: IPE

15:07 pm

Helps if you load the tray...!

15:52 pm 6 hour start PMA

4:28 cells spin

4:55 cells b/c

5:10 wash

5:20 1/2 min se

662 - 377 = 285

Extra @ 5:43pm

6:03

sc's 5:47 spin

sc's Inc 6:18pm

Fix Perm 6:53pm

8:11 pm first batch

8:21pm done

End 4/0: 22:08

Extra cell 2nd: 10:39pm

cell fix Perm: 23:18pm

Intra ne 00:00

Done @ 12:53 am

Ex vivo Gd-p1 X 4.5					Ex vivo Gd-p2 X 4.5				
V450 BV421	PD1	2.0	9.0		BV421	CD3	1.5	6.8	
V525 BV510	Aqua	1:500	-		BV510	CD69	2.0	9.0	
V670 BV650	CD16	1.5	6.8		BV650	CD56	1.8	8.1	
B530 FITC	Vd2	1.2	5.4		FITC	Vd2	1.2	5.4	
B710									
PerCPeF710	CD25	2.0	9.0		PerCPeF710	Perforin	-	3.0	13.5
Y590 PE	CD28	2uL	9.0		PE	NKG2D	0.6	2.7	
Y615					PE/Dazzle™				
PE/Dazzle™ 594	CD27	1.5	6.8		<del>594</del>	<del>CD57</del>	<del>1.5</del>	<del>6.8</del>	
Y780 PE									
Vio770	CD3	0.5ul	2.25		PE Vio770	NKG2A	0.6	2.7	
R670 APC	Vd1	1ul	4.5		APC	Vd1	1ul	4.5	
R780									
APC Fire750	CD45RA	1.8	8.1		APCCy7	Horizon™ 780	1:1000	-	
20ul/rxn;		7ul	31.5		20ul/rxn;		10.3 ul	46.4	17.5 78.8

p1 Vd1  
 CD3 Fite 1.5  
 APC CD27 1.5  
 PE Vio770 1ul  
 20ul/rxn  
 p1 Vd2  
 Vd2 Fite 1.2  
 APC CD27 1.5

Not in it

PMA: (2 pl PMA \* 500 ml media) \* # samples. + cells (1.5) + media 1500 pl

800  
x 17 Aqua

13.6 ml PBS ~ 34 pl L/A

Horizon 4 ml ~ 4 pl L/A

15  
x 5  
7500 ml H<sub>2</sub>O  
75

6.750 ml + 750 pl RBC  
H<sub>2</sub>O 10x

R10  
40 ml media  
+ 16 ml resuspension  
+ volume reser  
+ resuspension volume  
cell res  
(last week today)

150 ml ~ 135 RBC  
1.5 G/L  
15 ml FBS  
150 pl Certogen

FBS - 10% ← 60 ml  
20 ml - 180 ml  
10 90  
15 - 135 ml

1.5 Permeash

4 x 36

6 ml 54 ml

FBS - PBS  
800 - 8 ml 2-2

.3 \* 20 = 3.3  
~ 4.3 ml

BV425

PD1 + PE not well diff

\* 615 issue previous run.

per Chris.

Word needed on pop g-las

← compensation on the LSR-II

9-11 am arrived 9:30 bumped @ 10:30 ~ 1 hour

6:30-9 pm 6:30-7:30 Chris; ~ 1 1/2 hour

8:30-10 am last 2 sets?

+ VDI panel 7:30 min

18:30 AM