April 30th, 2021

HEU: γδ panel optimizations

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+M	Total	1.5E+6	2E+6
NY058			10^7	12-16-15							
CQ957							3.6 E6	5.0E6			

Test Version	Ex vivo Gd-p4b	Surface µL	x	Intra µL	x
BV421	PD1	2			
BV510	CD26	1.5			
BV650	CD56	1.8			
Alexa 488 (FITC)	dg9	-		3	
PerCPeF710	CD3	1.5			
PE	GrzmB	-		1.5	
PE Dazzle	-				
PE Vio770	NKG2A	0.6			
APC	VD2	1.0			
APC Fire750	Horizon L/D	1:1000			
20.5 µl/rxn;	PBS	12.1		16	

	Ex vivo Gd-p2	Surface µL	x	Intra µL	x
BV421	VD2	1			
BV510	Aqua L/D	1:500			
BV650	CD56	1.8			
Alexa 488 (FITC)	dg9	-		3	
PerCPeF710	CD3	1.5			
PE	NKG2D	036			
PE Dazzle					
PE Vio770	NKG2A	0.6			
APC	VD1	1.0			
APC Fire750	CD69	2.0			
20.5 µl/rxn;	PBS	12.0		17.5	

Test Version B	Ex vivo Gd-CK PMA	Surface µL	x	Intra µL	x
BV421	PD1	2			
BV510	Aqua L/D	1:500			
BV650	CD27	1.5			
FITC	VD2	1.2		0.2	
PerCPeF710	CD3	1		0.2	
PE	CD56	0.5			
PE Dazzle					
PE Vio770	IFNg			0.6	
Alexa 647 (APC)	TNFa			2	
APC Fire750	CD45RO	2			
20.5 µl/rxn;	PBS	12.3		17.5	

Test Version C	Ex vivo Gd-CK PMA	Surface µL	x	Intra µL	x
BV421	PD1	2			
BV510	CD45RA	1.8			
BV650	CD27	1.5			
FITC	VD2	1.2		0.2	
PerCPeF710	CD3	1		0.2	
PE	CD56	0.5			
PE Dazzle					
PE Vio770	INFg			0.6	
Alexa 647 (APC)	TNFa			2	
APC Fire750	L/D	1:1000			
20.5 µl/rxn;					

David Rad

FSC → 550
10:30 start collection

04/30/2021

		PHB	×1		
BV421	PD1	2.0	—		2.4 ml so 2.4 µl Horizon
BUS10	CD26(M-A261)	1.5	—		2.4 ml so 4.8 µl 1/2 Aqua.
Stop BU650	CD56	~~1.8~~	~~—~~		
FITC	dg9	—	3		L/D @ 15:58 → 16:13
PerCPef710	CD3	1.5	—		4:26 single colors in → 4:44
PE	GRMb	—	~~2~~ (1.5)		4:48 1/4 RBC 1/2 sc 04:51
—					5:02 golgi
PEVio770	NKG2A	0.6	—		5:43 Ice on ice
Alex647	VD2	1.0	—		Done @ 6:34
APC-Fire	L/D	1:1000			
	PBS	12.1	16		

N4058 10^7 12-16-15

Sc stain @ 20:19
@ 20:55 sc spin

	P2	×1		
BV421	VD2	~~1.0~~	—	9:34 scs out.
BUS10	L/D	1:500	—	
BU650	CD56	1.8	—	L/D @ 21:53 → 10:08
FITC	dg9	—	3	
PerCP	CD3	1.5	—	Cells frothed @ 2ml FBS addition...
PE	NKG2D	0.6	—	Surface @ 10:23 → 10:43
—		—	—	RBC @ 10:45 ✓ → :48
PEVio770	NKG2A	0.6	—	Fix Perm @ 11:00
Alex647	VD1	1.0	—	Intracellular @ 23:40 → 12:20
APC Fire	CD69	2.0	—	
	PBS	12.0	~~12.5~~	Done @ 12:00 ?

12:30 HHSO throw
15:39 PMA in
15:46 start flow

PMA/Ctrl
4µl 1ml
2.4 coop

TT well @ 250 µl ~ 71M Lym
60M 120 µl control + 180
1M 200 µl PMA + 300
240 µl P2 + Pb

B)

	Cha		× 2.5			
BV421	PD1		2	5	—	
BUS10	L/D		1:500		—	
BUG50	CD27		1.5	3.8	—	
FITC	~~CD3~~ VD2		1.2	3	.2	.5
PerCpCef710	~~CD56~~ CD3		1.	2.5	.2	.5
PE	CD56		.5	1.25	—	
—			—	-	—	
PeVio770	IFNg		—	-	0.6	1.5
Alex647	TNFa		—	-	2	5.0
APC Fire750	CD45RO		2	5		
	~~For~~ PBS		12.3	30.8	17.5	35.0

CQ957 Lym = 8E+5
1.5ml 5E+6/ml
Ly = 3.6E+6/ml

Gating on living cells would also help.

Les
Larissa
Lilith
Lesl.e

C)

		× 2.5			
BV421	PD1		2	5	—
BUS10	CD45RA		1.0	4.5	—
BUG50	CD27		1.5	3.8	—
~~FITC~~ VD2			1.2	3	.2 .5
PerCp	CD3		1.0	2.5	.2 .5
PE	CD56		.5	1.25	—
—			—	—	—
PeVio7	IFNg		—	-	.6 1.5
Alex647	TNFa		—	-	2 5.0
APC Fire750	L/D		1:1000		
	PBS		12.5	31.25	17.5 35.0