+ CXCR5 R780 test

PPD & Icos vs. Golgi Addition

January 21st, 2025

Specimen	Status	Location	Conc	Date	Notes	Volume	CD45+	Total	Resuspension
NY41	Old Adult		25E6	2-15-12	Nitrogen Inside	2	4.9E6	9.8	↑1.3 (1.6)
CVD 6022	PPD+ Adult		15E6	3-9-23		2	8.0E6	16.0	↑3.3

1:41 pm Thaw Start
NY041 had LN2 !!
1:51 pm spin
2:05 pm DNAse
2:15 pm stain Count
Incubation @ 2:45 pm

8:59 PPD AND CD107a added
(4 µL) (0.96) µL)
780 µL volume = 2.34 M cells + CVD6022 80 µL unstained ≈ 240 K cells

One NY041 and 1 CVD6022 still Pink !!
Making it a ctrl
And making it the golgi immediate

ctrl SEB
5 µL
15 × 5 = 75 µL
Extra
0 1 2 3 4
3M 3M 3M 3M 3M
300
30 µL
Intra
2M
An unstained is critical!

+ CD107a
5 µL × .66 = 3.30 µL PPD × 6 = 19.8 µL PPD

1.2 × .66 = 720 + 72 = 0.792 µL

9 AM start in the AM ✓
- Golgi 0 @ 8:59 pm ✓
- Golgi 1 @ <9:59 pm> ✓
- Golgi 2 surf @ <10:59 pm> ✓
  2 intra @ <10:59 pm> ✓
- Golgi 3 @ <11:59 pm> ✓
- Golgi 4 @ <12:59 pm>

0.800 × 5 = 4 µL

(PPD stim)
(PPD retention)
(Icos surface)

1.2 × .8 = 0.96

Ab prep @ 8:45 AM
9:13 AM spin
9:30 AM zombie spin 9:48 AM
9:36 Hot sds spun 1400 8
9:46 cold sds spun 1400 8 @ 10:20 ✓
10:01 samples Hot
No icos @ this point

10:36 AM wash max & made
10:49 AM Samples Cold → 11:19 AM spin @ 11:22 am
10:54 AM sc Fix → 04 → 14 → 24
11:30 AM strept → 11:45 AM
11:58 am → 08 → 18 → 12:28
12:01 pm sds Intra → 12:40
Samples Intra @ 12:52 pm → 1:32 pm

spin @ 11:50
1st wash @ 12:28 pm ✓
2nd wash @ 12:36 am ✓
3rd @ 1:34 pm
4th @ 1:42 p
Done @ 1:53

50 µL Volume PFA-PBS 0.4% to all sds

300 µl scs Fix

× 36

10,800

600 µl for samples

× 6

3.6 mL

Fix

25-75

10.8

3.6

15.0

3.75 Fix

11.25 Diluent

600 µl / wash

× 4

2.4 mL

× 4

9.6 mL

× 2

1.2 ml

32

2.4

3 6.0

38.4 mL

28.8 mL

9.6 mL

76.8 mL wash

1.2 ml wash

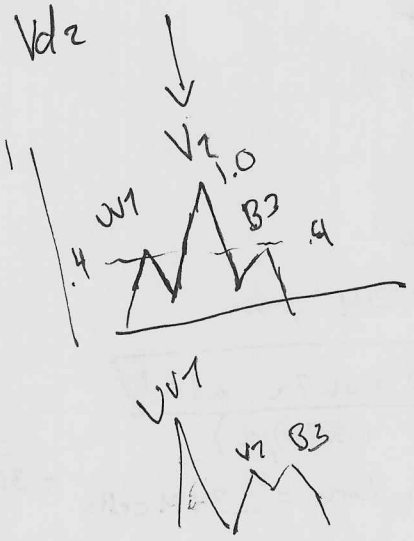
× 4

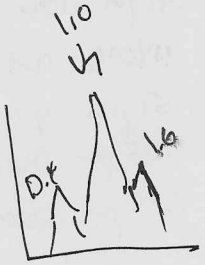
4.8 ml / sample

× 6

28.8 mls

8mL + 72 mL H2O





Time 0	0.69	0.03
Time 1	0.89	0.02
Time 2	1.27	0.03 ← Surface
	- 1.17	← 1.51 intrac
Time 3	1.60	0.02
Time 4	1.81	0.02

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αβ T cell SFC panel

	#	Filter	Fluorochrome	Marker	Clone	Dose	During stim!!!	6	L/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	6	ColdStain 30min @4C	6	4*C for 15 min	RBC Lyse, then Fix/Perm	Intranuclear Stain 40 min @RT	6
1		UV1																
		UV2	BUV395	CD62L	(DREG-56)	1							1.2	7.2				
		UV6																
2	1	UV7																
	1	UV9	BUV563	CD69	(FN50)	2												
3		UV10	BUV615	CCR4	(1G1)	2					1.8	10.8					0.8	4.8
4		UV11	BUV661	Vδ2	(B6)	2							0.5	3				
5	2	UV14	BUV737	CD38		1							0.5	3			0.1	0.6
6	3	UV16	BUV805	CD4	(SK3)	1					1.5	9						
		V1	BV421/														0.2	1.2
		V2																
7	4	V3	Pacific Blue	CD14	(M5E2)	2							1.5	9				
		V3	Pacific Blue	CD19	(HIB19)								1.5	9				
		V4																
8	5	V5	BV480	CD161	(REA631)	1					1.5	9			Strep <0.4>			
		V6																
		V7																
9		V8	BV570	CD16		1							0.9	5.4				
		V9																
10	6	V10	BV605	CD45RA	(J252D4)	1							0.9	5.4				
11	7	V11	BV650	CD8		1							1.0	6				
12		V13	BV711	Va7.2		2							1.2	7.2				
13	8	V14	BV750	IFNγ	(B27)	2												
14		V15	BV786	CCR6	(11A9)	1					1.2	7.2					1.5	9
		V16																
15		B2	Alexa Fluor 488	FoxP3		Beads												
16	9	B3	Spark Blue 550	CD3	(SK7)	1											3	18
17	10	B4	Spark Blue 574	CD45		1							1.2	7.2			0.2	1.2
18	11	B6	RB613	PD1		1							0.6	3.6				
		B7									1	6						
		B8																
		B9																
19		B10	RB705	CD26	(M-A261)	1												
		B12											1.0	6				
		B13																
20		B14	RB780	CXCR5?		2												
21	12	YG1	PE	ICOS	(EH12.2H7	1					1.2	7.2						
		YG2									<>1	######						
22	13	YG3	PE-Dazzle 594	TNFα	(MAB11)	2												
		YG4																
23		YG5	PE-Cy5	CXCR3		2											1	6
		YG6									1	6						
24		YG7	PE-Fire 700	CD127		1												
25	14	YG8	PE-Fire 744	CD25		2							1.5	9				
26		YG9	PE-Vio 770	HLA-DR	(L243)	1					1.2	7.2						
		YG10	PE-Fire810	CD56?		1							0.6	3.6				
27		R1	APC	CD39	(A1)	1												
28	15	R2	Alexa Fluor 647	IL2	(MQ1-	2					1.2	7.2						
		R3																
29	16	R4	APC-R700	CD107a	(H4A3)	Supernatan	0.6	3.6									1.2	7.2
		R5																
30	17	R6	Zombie NIR	L/D		2			<1:2500>									
31		R7	APC-Fire 750	CD27	(O323)	1												
32	18	R8	APC-Fire 810	CCR7		1					1	6	1.5	9				
	And UNSTAINED CONTROLS !!!				Antibody Total	0.6			Antibody Total		12.6	75.6	15.6	93.6			8	48
					R10 Media	19.9			Brilliant Stain		25.2	151.2	31.2	187.2			16	96
					Pippette draw volume	19.5			Pippette draw		34.8		43.8				21	

Wash with 2 ml PBS, spin down 1300rpm 8min
800 ul of LiveDead mix (1:2500) @RT for 15min
Wash 1.5 ml 5% PBS-FBS, spin 1300 rpm, 8min

Add CD161 biotin antibody for 10 minutes at RT

Add HotStain mix, incubate @ 37C for 30min
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min

Add ColdStain mix, incubate @ 4C for 30min
Add 300-500 ul 1x RBC Lysis for 3 minutes
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min

Add Streptavidin Mix, incubate @ 4C for 15min
Wash 1.5 ml 5% PBS-FBS 1400 rpm, 6min

0.8 ml Nuclear FixPerm, incubate @ 4C for 30min
(vortex every 10 minutes)

First PermWash: 1.5 ml NuclearWash 1500 rpm 6 min

Second Perm Wash: 1.5 ml NuclearWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min
First PermWash: 1.5 ml NuclearWash 1500 rpm 6 min
Second Perm Wash: 1.5 ml NuclearWash 1500 rpm 6 min

Resuspend in 80 ul 0.4% PFA-PBS
Cap tubes, wrap rack in foil, store at 4*C

RF

32 = 5 doses

5.1H1L CD56 BrA19 CL405-65264 Coralite

Alexa Fluor 405
Dylight 405
Spark Violet 423
Super Bright 436
VioBright 423

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Inf 914-4 HU ♂ 41 wks 2022-02-10

2022-06-16

↑ 3 up 2 over

12A Row G, Col 2

(12B Row A Col 2)