

December 8th, Cyto Abstract

24th
November 23rd, 2024

Specimen	Status	Location	Conc	Date	Notes	Volume	CD45+	Total	Resuspension
NY026	Adult		20E+6	7/26/2011		3 mL	7.8E6	23.4	↑4.7

Prep Work @ 6:02 pm

Regulate Prep @ 7:00 pm

31 doses

Thaw @ 7:10 pm

Spin @ 7:16 pm

DNase @ 7:30 pm

Aliquoting @ 7:57 pm

Spin @ 8:10 pm

Wash @ 8:23 Spin @ 8:41 pm

Hot SC's @ 8:31 pm spin @ 8:50 pm (shaken at 20)

Cold SC's @ 8:37 pm → spin @ 9:08 pm

FS Hot @ 8:58 pm → 9:18 pm spin @ 9:19

SC's Fix @ 9:22 → 32:42:52 pm

FS cold @ 9:32 pm → 10:02 pm

→ Spin @ 10:09

✓ 2nd Pen Wash SC's

Strept @ 10:20 pm

SC's done @ 10:29 pm

10:38 pm Streptawash spin

10:47 pm Fix Samples → 57 → 07 → 17 pm

11:30 pm 2nd Pen Wash FS

Done @ 11:36 pm

2 amber
Icos

CD3
CD4
CD8
CD45

0.8

0.3

0.03

0.8 300 = 15.0 ML

660
15
3300

7.700

8.300

4.400

6.2

1.80

PE Icos 0.8 µL 0.08 µL 0.008 µL

Just fill mixers
titrations to
fridge

Old
BUH21 1.5 µL
CD127
SBY720
CD45RA ↑
1.5 µL
SB574
CD45
0.8 µL

New
BUH21
old CD45 RA
10 µL
PF F2700
CD127 µL
SB574
old CD45
0.8 µL

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Date		Time		Location		Weather		Notes	

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αβ T cell SFC panel																
#	Filter	Fluorochrome	Marker	Clone	Dose	During stim [i]	0	L/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	2	Cold Stain 30min @4C	2	4°C for 15 min	Rac Lys, then FixPerm	Intranuclear Stain 40 min @RT
UV2		BUV395	CD62L	(DREG-56)	1							1.2	2.4			0
UV7		BUV563	CD69	(FN50)	2											
UV10		BUV615	CCR4 (1G1)		2					1.8	3.6					0.8
UV11		BUV661	Vβ2 (B6)		2							0.5	1			0
UV14		BUV737	CD38	(SK3)	1							0.5	1			0
UV16		BUV805	CD4	(SK3)	1											
V1		BU421	CD127?		1					1.5	3					0.7
V3		Pacific Blue	CD14 (M5E2)		2					1.5		1.5	3			0
V3		Pacific Blue	CD19 (HIB19)									1.5	3			
V5		BU480	CD161 (RE4631)		1					1.5	3			Strep <0.45		
V7		BU510	CD45RA?	(H100)	1											
V8		BU570														
V10		BU605	CKCR5/CD45RA	(J252D4)	2					2.5		1.0	2.4			1.5
V11		BU650	CD8?		2											0
V13		BU711	Vα7/2		1							1.2	2.4			0.5
V14		BU750	IFNγ	(B27)	2											
V15		BU786	CCR6	(11A9)	1											
B2		Alexa Fluor 488	FoxP3		1					1.2	2.4					0
B3		Spark Blue 550	CD3	(SK7)	1											
B4		Spark Blue 574	CD45?		1											
B6		RB613	PD1		1							1.2	2.4			3
B9		RB705	CD26?	(M-A261)	1					1	2					0
B14		RB780	CKCR5?		1											
YG1		PE	ICOS?	(EH12.2H7.1)	1											2
YG3		PE-Dazzle 594	TNFR	(MA811)	2					0.8						0
YG5		PE-Cy5	CKCR3		2											
YG7		PE-Fire 700	CD127?	CD45RA	2					1	2	1.5				1
YG8		PE-Fire 744	CD25		1											0
YG9		PE-Vio 770	H1A-DR	(L243)	1					1.2	2.4					
YG10		PE-Fire810			1							0.6	1.2			
R1		APC	CD39	(A1)	1											
R2		Alexa Fluor 647	IL2	(MO1)	2					1.2	2.4					
R4		APC-R700	CD107a	(H4A3)	1											1.2
R6		Zombie NIR	L/D	Supernatant	1.2	0										0
R7		APC-Fire 750	CD27	(O323)	2											
R8		APC-Fire 810	CCR7		1							1.5	3			
And UNSTAINED CONTROLS !!!																
						Antibody Total	1.2	Antibody Total	16.2	2	32.4	9.7	19.4	10.8		
						R10 Media	19.3	Brilliant Stain	32.4	64.8	19.4	38.8	21.6	0		
						Pipette draw volume	19.5	Pipette draw	45.6	26.1			29.4	0		

Wash with 2 ml PBS, spin down 1300rpm 8min
800 ul of Live/Dead 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

27 SCS * 1.5 = 40.5
5 FS * 6.0 = 30.0
70.5 FBS-FBS

NY026

27 * 1.5 = 40.5
5 * 30 = 150
56.5 Perm Wash
27 * 400 = 10,800 ml
5 * 800 = 400 ml
15,000 ml Fix

fix 2 = 4.8 ml Zombie NIR

20 FL
22011

11

10

8

2

2

11

10

11
10
8

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