

November 18<sup>th</sup>

Not exCP5 or CD127 being old

Specimen	Status	Location	Conc	Date	Notes	Volume	CD45+	Total	Resuspension
NY071			15E+6			1ml	14E+9		↑6mL

Ab prep @ 9:42 am → 10:12 am

Reagents @ 10:34 am

Thaw @ 10:46 am

Spin @ 10:50 am

INT sc @ 11:04 am

Spin @ 11:16 am

W/O @ 11:28 am @ 11:44 spin

Hot sc's @ 11:31 12:09 ✓

Cold sc's @ 11:42 12:14 ✓

Hot FS @ 11:58 pm @ 12:29 spin

12:41 Cold FS @ 1:11 pm

3rd SC vortex @ 1:06 pm

Fix SC's @ 12:36 → 56 ✓  
Spin @ 1:07 pm

FS @ strap wash @ 1:40 pm

+ BD161 BV480 fixed & permeabil

twice then strap then permeabil again  
(cups!)

#15  
Spin wash f.s @ 2:22 pm

- 2 mL = 5 mL ~ 10 mL ↑ 6.8 mL  
- 200 μL

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$$27 \times 5 \text{ CS} \times 1.5 = 40$$

$$2 \text{ fs} \times 6.0 = 12.0$$

12.0mls FBS-PBS

$$1 \times 2 \text{ washes} \times 1.5 = 40 \text{ ml}$$

0ml

40 ml Perm Wash

$$27 \times 400 = 108 \text{ ml}$$

$$2 \times 800$$

$$1.6$$

124ml Fix

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q3) T cell SFC panel

#	Filter	Fluorochrome	Marker	Clone	Dose	During stim!!!	0	L/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	2	ColdStain 30min @4C	2	4°C for 15 min	RBC Lys, then FixPerm	Intranuclear Stain 40 min @RT	0
UV2		BUV395	CD62L	(DMEG-56)	1							1.2	2.4				
UV9		BUV563	CD69	(FN50)	2												
UV10		BUV615	CCR4	(161)	2					1.8	3.6	0.5	1			0.8	0
UV11		BUV661	VR2	(B6)	2							0.5	1			0.1	0
UV14		BUV737	CD38		1							0.5	1				
UV16		BUV805	CD4	(SK3)	1					1.5	3					0.7	0
V1		BUV421	CD127		1					1.5		1.5	3				
V3		Pacific Blue	CD14	(M5E2)	2							1.5	3				
V3		Pacific Blue	CD19	(H1B19)	2							1.5	3				
V5		BUV480	CD161	(REA631)	1					1.5	3			Strept			
V7		BUV480	CD161	(REA631)	1									<0.4>			
V8		BUV570	CD45RO	(H1101)	2												
V10		BUV605	CD45RO	(J252D4)	2					2.5							
V13		BUV711	VR7.2		2							1.2	2.4			1.5	0
V14		BUV750	IFNV	(B27)	2												
V15		BUV786	CCR6	(11A9)	1					1.2	2.4						
B2		Alexa Fluor 488	FoxP3		1							1.2	2.4			3	0
B3		Spark Blue 550	CD3	(SK7)	1							1.2	2.4			0.5	0
B4		Spark Blue 574	CD8		1							1.0	2				
B6		RB613	PD1		1					1	2						
B9		PerCP-Cy5.5	CD26	(M-A261)	1											2	0
B14		StarBright 816			1												
YG1		PE	ICOS	(EH12.2H7)	1					0.8	1.6						
YG3		PE-Dazzle 594	TNFR	(MA811)	2												
YG5		PE-Cy5	CD45RA		2					1	2	1.0	2			1	0
YG7		StarBright 7720	CD25		1												
YG8		PE-Fire 744	CD25		1					1.2	2.4						
YG9		PE-Mio 770	HLA-DR	(1243)	1							0.6	1.2				
YG10		PE-Fire 810			1												
R1		APC	CD39	(A1)	1												
R2		Alexa Fluor 647	IL2	(MO1-2)	1					1.2	2.4					1.2	0
R4		APC-R700	CD107a	(H4A3)	2												
R6		Zombie NIR	L/D	Supernatant	2												
R7		APC-Fire 750	CD27	(O323)	1							1.5	3				
R8		APC-Fire 810	CD27		1												
And UNSTAINED CONTROLS !!!																	
					Antibody Total	1.2				Antibody Total	16.2	32.4	11.7	23.4		10.8	0
					R10 Media	19.3				Brilliant Stain	32.4	64.8	23.4	46.8		21.6	0
					Pipette draw volume	19.5				Pipette draw	45.6		32.1			29.4	0

34 doses

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 HoeStain 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



November 18<sup>th</sup>, 2024

1. 10/10/2024  
2. 10/10/2024  
3. 10/10/2024  
4. 10/10/2024  
5. 10/10/2024

November 18<sup>th</sup>, 2024

5L<sup>o</sup>  
Yellow Laser Delay  
Red Laser Delay  
C5<sup>o</sup> Yelw-Green Delay  
Red Delay  
4L Red Delay  
3L Red Delay