

April 24th, 2025

Specimen	Status	Location	Conc	Date	Notes	Volume	CD45+	Total	Resuspension
ND050 a1			12 EC	2/13/25				9.3	
ND050 a2			12 EC					8.3	
ND044 a1			10 EC	2/27/13	A Handwritten			6	
ND044 a2			10 EC	2/27/13				12	

11:45 AM then start

12:13 pm DNAse

12:20 pm stain for count

13:08 stain count

7:10 spin

7:52 mixes made

8:34 spin after about

4:00 9:11 pm

1st test 9:18 pm

Cold 9:28 pm spin 31

samples Hct: 9:52 pm → 10:22

10:35 pm cold samples (ND044 PMA missing 4°C Ab)

11:07 pm spin

Fix 11:20 pm

11:45 pm spin #1 12:04 pm #2nd spin

12:23 pm Intra

3rd @ 1:06 am

Done 1:28 AM

Per CP-cy5.5 dg9

$$ND050 \quad 17.6/6 = \underline{\underline{2.93}}$$

$$ND044 \quad 18.0/6 = \underline{\underline{3.00}}$$

April 24th, 2025

10:00 am

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#	Filter	Fluorochrome	Marker	Clone	Dose	During stim!!!	3	L/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	3	Cold Stain 30min @4C	3	4°C for 15 min	RBC Lysis, then FixPerm	Intranuclear Stain 40 min @RT	3
1	UV1	BUV395	CD62L	(DREG-56)	1							1.2	3.6				
2	UV2	BUV563	CD69	(FN50)	2												
3	UV10	BUV615	CD4	(1G1)	2					1.8	5.4	0.5	1.5			<0.8>	#####
4	UV11	BUV661	CD38	(86)	2							<0.5>	#####			0.1	0.3
5	UV14	BUV737	CD4	(S13)	1					<1.5>	#####					0.2	0.6
6	UV16	BUV805	CD4	(S13)	1												
7	UV2	BUV421															
8	UV3	Pacific Blue	CD14	(M5E2)	2							1.5	4.5				
9	UV4	Pacific Blue	CD19	(HIB19)	2							1.5	4.5				
10	UV5	BUV480	CD161	(REA631)	1					1.5	4.5			Strep <0.4>			
11	UV6	BUV570	CD16		1							0.9	2.7				
12	UV9	BUV605	CD45RA	(J25/D4)	1							0.9	2.7				
13	UV11	BUV650	CD8		1							1.0	3				
14	UV14	BUV711	IFN γ	(827)	2							1.2	3.6				
15	UV15	BUV750	CCR6	(1A9)	1					1.2	3.6					1.5	4.5
16	UV16	Alexa Fluor 488	CD3														
17	B2	Spark Blue S50	CD3	(SK7)	1							1.2	3.6			3	9
18	B4	Spark Blue S74	CD45		1							0.6	1.8			0.2	0.6
19	B6	RB613	PD1		1					1	3						
20	B10	RB705	CD26	(M-A261)	1							1.0	3				
21	B14	RB780	CD45		2							1.2	3.6				
22	YG1	PE	ICOS	(EH12.2H7.1)	2												
23	YG2	PE-Dazzle 594	TNFR	(MAB11)	2											1.5	4.5
24	YG3	PE-Cy5	CD3		2					1	3					1	3
25	YG6	PE-Fire 700	CD127		1							1.5	4.5				
26	YG8	PE-Fire 744	CD25		2					1.2	3.6						
27	YG9	PE-Vio 770	HLA-DR	(1243)	1							0.6	1.8				
28	YG10	APC	CD39	(A11)	1					1.2	3.6						
29	R1	Alexa Fluor 647	IL2	(MO1-2)	1												
30	R2																
31	R3															1.2	3.6
32	R4																
33	R5	Zombie NIR	L/D		2												
34	R6	APC-Fire 750	CD27	(O323)	1					<1.2500>		1.5	4.5				
And UNSTAINED CONTROLS !!!																	
Antibody Total						0.0	Antibody Total		8.9	26.7	16.3	48.9	Antibody Total		8.7	26.1	
R10 Media						20.5	Brilliant Stain		17.8	53.4	32.6	97.8	Brilliant Stain		17.4	52.2	
Pipette draw volume						19.5	Pipette draw		23.7	45.9			Pipette draw		23.1		

cd3 T cell SFC panel

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 Mx, 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 50 ul 0.4% PFA+PBS
Cap tubes, wrap rack in foil, store at 4°C

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14 5/15

