

September 13<sup>th</sup>, 2024

Test       

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
ND044									

5:07 pm Antigen 2  
CD107a (LSpl) in

7:09 pm Cells + golgi In

September 14<sup>th</sup>, 2024

7:04 am cells out

7:08 am cells spin

7:47 am Antibodies spin

8:33 am Reagents prepped

9:20 am Zombie

9:28 AM 37° scs

9:36 AM 4° sc's

9:48 AM Samples Hot

10:30 cold samples

11:03 RBC lyse

11:15 strept

11:45 Fix

12:50 Intrae

12:55 sc's done

1:34 3'd wash

Sept 14<sup>th</sup>, 2024

Acquisition @ 3:12 pm

September 13<sup>th</sup>, 2024

Date		Time		Location		Notes	

September 13th, 2024

αβ T cell SFC panel																	
#	Filter	Fluorochrome	Marker	Clone	Vial lot #	During stim!!!	2	L/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	2	Cold Stain 30min @4C	2	4°C for 15 min	Rec Lys, then FixPerm	Intracellular Stain 40 min @RT	2
1	UV2	BUV395	CD62L	(DREG-56)								1.2	2.4				
2	UV7	BUV496	CD8	(RPA-T8)								0.7	1.4				
3	UV9	BUV563	CD69	(FN50)													
4	UV10	BUV615	CCR4	(1G1)						1.8						0.8	1.6
5	UV11	BUV661	Vβ2	(B6)													
6	UV14	BUV737	CD38									0.5	1			0.1	0.2
7	UV16	BUV805	CD4	(SK3)								0.5	1				
8	V1	BUV421	Vα7.2							1.5		3				0.7	1.4
V3		Pacific Blue	CD14	(MSE2)								1.2	2.4				
9	V5	BUV480	CD161	(REA631)						1.5		3					
10	V7	BUV510	CD45RA	(HI100)								1.5	3				
11	V10	BUV605	CD45RA	(HI100)								1.5	3				
12	V11	BUV650	CD45RA	(J252D4)								0.5	1		Strep <0.8>		
13	V13	BUV711	ICOS	(3C10)						2.5		5					
13	V14	BUV750	IFNγ	(B27)								1.2	2.4				
14	V15	BUV786	CCR6	(11A9)													
15	B2	Alexa Fluor 488	FoxP3							1.2		2.4				1.5	3
16	B3	Spark Blue 550	CD3	(SK7)													
17	B9	PerCP-Cy5.5	CD26	(M-A261)								1.2	2.4			3	6
18	VG1	PE	PD1	(EH12.2H7)												0.5	1
19	VG3	PE-Dazzle594	TNFr	(MA811)								1.0	2			1.5	3
20	VG5	PE-Cy5	CD25	(M-A251)												1.2	2.4
21	VG9	cFluor-BV6750								1.2		2.4					
22	VG10	PE-wig770	HLA-DR	(1243)								0.8	1.6				
23	R1	PE-Fire810	CCR7									0.5	1				
24	R2	APC	CD39	(A1)						1.2		2.4					
25	R4	Alexa Fluor 647	IL2	(M01-)													
26	R6	APC-R700	CD107a	(H4A3)												1.2	2.4
27	R7	Zombie NIR	L/D							3							
28	R8	APC/Fire 750	CD27	(O323)													
APC/Fire 810												1.5	3				
And UNSTAINED CONTROLS !!!																	
Antibody Total						1.5				Antibody Total	10.9	21.8	13.8	27.6		10.5	21
R10 Media						19.0				Brilliant Stain	21.8	43.6	27.6	55.2		21	42
Pipette draw volume						19.5				Pipette draw	29.7		38.4			28.5	

Wash with 1 ml PBS, spin down 1300rpm 8min  
 800 ul of LiveDead mix (1:2500) @RT for 15min  
 Wash 2 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 2 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 2 ml 5% PBS-FBS 1400 rpm, 6min

Add Streptavidin Mx, incubate @ 4C for 15min  
 Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

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

First PermWash: 2 ml NuclearWash 1500 rpm 6 min  
 Second Perm Wash: 2 ml NuclearWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min  
 First PermWash: 2 ml NuclearWash 1500 rpm 6 min  
 Second Perm Wash: 2 ml NuclearWash 1500 rpm 6 min

Resuspend in 30 ul 0.4% PFA-PBS  
 Cap tubes, wrap rack in foil, store at 4°C



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