

September 6th, 2024

CUD6022 PPD

Specimen	Status	Location	Conc	Date	Notes	Volume	Lym	Lym+Mon	Total
CUD6022	PPD+ Nigerian Adult ♂		15EG	3/9/23					
NY068	Adult PPD+		15EG	8/15/23					

Sept 6th, 2024 Friday

9:08 AM start

9:32 AM DNase 30pl

9:47 AM

10:01 AM Count Done

10:40 AM Rest start 4x

3:47 pm stim Added

3:54 pm 2 pl CD107a added

5:58 pm Golgi Added

9:42 Cold wash

9:52 SC's wash

9:55 stop wash Fill

→ wash @ 10:15

10:20 SC intra

10:24 Samples fix → 54

10:37 Samples Perm

11:22 Samples Intracellular

12:12 Final wash

Done @ 12:22pm

resuspension in 80pl

Sept 7th, 2024 Saturday

5:49 AM Cells out

5:57 am Samples spin

6:11 am Samples Fridge

6:18 am SC's spin

6:35 am Stain prep

→ 7:05

→ 7:40

7:44 am L/D stain

7:59 am Cold SC's? (8:34 wash)

8:01 am L/D spin

8:12 am Hot SC's (8:50 wash)

8:18 am Hot Full Intra

9:05 cold Full stain

9:12 Fix for SC's → 9:42

9:39 RBC lysis

September 6th, 2024

#	Filter	Fluorochrome	Marker	Clone	Vial Lot #	During stimuli	8	L/D RT 15 min	RT for 10 min	Hot Stain @4C 30min	8	Cold Stain @4C 30min	8	4°C for 15 min	β T cell SFC panel RBC Lys, then FixPerm	titranuclear Stain 40 min @RT	8
1	UV2	BUV395	CD62L	(DREG-56)								1.2	9.6				
2	UV7	BUV496	CD8	(RPA-18)								0.7	5.6				
3	UV9	BUV563	CD69	(FMS5)													
4	UV10	BUV615	CCR6	(1G1)													
5	UV11	BUV661	VC2	(B6)						2	16	0.5	4			1	8
6	UV16	BUV737	CD4	(SK3)								1.3				0.1	0.8
7	V1	BUV805	CD3	(SK3)						1.5	12					0.5	4
8	V3	Pacific Blue	CD14	(MSE2)						1.5	12						
9	V3	Pacific Blue	CD19	(H1619)								1.5	12				
10	V5	BUV480	CD161	(RE4631)						1.5	12	1.5	12				
11	V10	BUV510	CD45RA	(H1100)								0.5	4	Strep <0.8>			
12	V11	BUV605	CD45	(J252D4)						2.5	20						
13	V13	BUV711	VC2	(3C10)								1.2	9.6			1.5	12
14	V14	BUV750	IFNγ	(B27)													
15	V15	BUV786	CCR6	(11A9)						1.5	12						
16	B3	Alexa Fluor 488	FoxP3														
17	B9	PerCP-Cy5.5	CD3	(SK7)								1.2	9.6			3	24
18	Y61	PE	PD1	(M-A261)								1.2	9.6			0.5	4
19	Y63	PE-Dazzle594	TNFA	(EH12.2H7)								1.2	9.6			0.1	0.8
20	Y65	PE-Cy5	CD25	(M-A251)												1.2	9.6
21	Y69	cFluor-BV6750								1.2	9.6					0.1	0.8
22	Y610	PE-vio770	HLA-DR	(L243)								0.8	6.4				
23	R1	PE-Fire810	CCR7	(A1)								1.0	<Add>			1	<Add>
24	R2	Alexa Fluor 647	IL2	(MO1-)						1.2	9.6						
25	R4	APC-R700	CD107a	(H4A3)													
26	R6	Zombie NIR	L/D													1.2	9.6
27	R7	APC/Fire 750	CD27	(0323)													
28	R8	APC/Fire 810	CD38	(HIT2)								1.5	12				
And UNSTAINED CONTROLS !!!				Antibody Total	0.0							1.5	12				
				R10 Media	20.5							15.6	124.8			12.2	97.6
				Pipette draw volume	19.5							31.2	249.6			24.4	195.2
				Antibody Total								43.8				33.6	
				Brilliant Stain													
				Pipette draw													

Wash with 1 ml PBS, spin down 1300rpm 6min
 800 ul of Live/Dead mix (1:2500) @RT for 15min
 Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 6min
 Add CD161 biotin antibody for 10 minutes at RT
 Add HoeStain 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 Mix, 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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