

December 28th, 2024

Specimen	Status	Location	Conc	Date	Notes	Volume	CD45+	Total	Resuspension
30μl ③ CUD6022	PPD+ Adult		15E6	3/9/23	22 remaining	2	9E6 18E6	18E6	↑4
30μl ① NY068	PPD+ Adult		15E6	8/5/23	27 remaining	2	10E6 20E6	21E6	↑4.7
30μl ② NY030	old Adult		20E6	10/19/11		2	2.4E6 50E6	17E6	↑3.7

+3L H₂O to 37°C water bath

really really loud rattling from the hood today.

Media appears grapefruit-ish...

10:35 AM Thaw start NY068

10:40 spin

11:15 stain for count

11:49 Rest start → 5:49 pm

4:39 pm recent stain 1.7 1.5 1.4

↳ We will not do NY031.

We will do CUD6022's 3 timepoints
plus NY068 5 time points.

NY031 for Icos titration if required

6:00 - 6:06 pm CD107a added,
PPD & SEB added

Volume is 500μl @ 3M → 1.5M

500μl @ 750K → 1.5M/mL

Next time don't increase volume!
maintain the conc (theoretically)

8:00 pm Add golgi block 5μl ~ 500

All added @ 8:00 pm ✓

10:42 Ab prep start

→ 11:25 pm Antibodies Made

11:50 pm Coffee ☺

3M → $\frac{0.75}{1.50}$ in 1mL

2.5
x11
25
250

27.5 μl PPD
& SEB

in 500μl vs in 1mL

5μg/mL → 2.5μg/500μl

3M/mL conc but 1.5E6 cells

+4 +6 +8 +10 +12
6, 8, 10, 12, 14

1.2 μl → 0.6 μl
per 500 μl

15
x0.6
9.0 μl

2.5
x8
20 μl PPD

25 μl SEB

0.6 CUD6022 1.5
x24 NY030 1.4
24
14.4 μl CD107a

125.6 R10

Act Act Act Act Act
CD69, TNFα, IFNγ, IL-2, CD107a
bends act act ctrl ctrl act
Icos, CD25, CD38, PD1

ctrl ctrl ctrl ctrl ctrl ctrl
CD3, CD4, CD8, CD161, PacBlue, CD45?

Zombie.

ctrl ctrl
CD45RA, CCR7

24/33

CUD6022 6 10 14
0 0 0
0 0 0

NY031 0 0 0
0 0 0

12+2 Act
12+2 ctrl

14
x.3
4.2 μl

		Mon	B cells	
182 [15] 21 [17.89]	2.3	CUD6022	5.67 HA	10.7
	3.8	NY068	11.6 HA	6.2
172 [9.69]	1.1	NY030	33.7	13.3

28-32

50% AB volumes & 50% Buffer

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12:1
SPK

Time 6 hrs (2+4)

Done

Spin: 11:59 pm

400pl Zombie 00:11 → 00:26

Hot 12:41 → 1:11 am spin @ 1:15 am

Cold 1:23 → 1:53 spin @ 1:59 am

Strept @ 2:11 am → 2:26 am spin @ 2:26

Fix @ 2:35 am → 4:5 → 5:5 → 3:05 am

1 spin @ 3:09 am

2 spin @ 3:18 am

Intra @ 3:27 am → 4:07 am

3 @ 4:09 am

4 @ 4:17 am / 4.18

0.4% PFA 50pl @ 4:29 am Wash

2 AM
Special

Time 8 hrs (2+6)

Done

Spin @ 1:59 am

400pl Zombie @ 2:08 am → 2:23 am spin @ 2:26

Hot @ 2:38 → 3:08 spin @ 3:09 am

Cold @ 3:20 → 3:50 am spin @ 3:50 am

Strept @ 3:58 am → 4:13 AM spin @ 4:17 am

Fix @ 4:26 → 3:5 → 4:5

1 @ 4:57 am ✓

2 @ 5:05 am ✓

Intra @ 5:12 am → 5:52 am

3 @ 5:55 am ✓

4 @ 6:03 am / 4.04

0.4% PFA 50pl @ 6:16 AM

4 AM
Special

(2+8)
Time 10 hrs

Done

Spin @ 4:00 AM

400pl Zombie @ 4:11 am → 4:26 spin @ 4:28

Hot @ 4:41 → 5:11 am spin @ 5:15 am

Cold @ 5:24 → 5:54 am spin @ 5:55 am

Strept @ 6:05 → 6:20 am spin @ 6:20

Fix @ 6:28 → 7:38 → 7:48 → 8:08 AM

1 @ 6:59 AM ✓

2 @ 7:08 AM ✓

Intra @ 7:17 → 7:57 AM

3 @ 8:03 AM

4 @ 8:12 AM / 4.12

0.4% PFA @ 8:26 AM ✓

6 AM
Special

Time 12 hrs

Done

Spin @ 6:03 am

400pl Zombie @ 6:14 → 6:29 spin @ 6:29 AM

Hot @ 6:42 → 7:12 AM spin @ 7:14 AM

Cold @ 7:23 → 7:53 AM spin @ 7:53 AM

Strept @ 8:02 → 8:17 AM spin @ 8:17 AM

Fix @ 8:28 → 8:44 am

1 → 8:54 → 9:04 → 9:14

2 → 9:17 am

3 → 9:26 am

Intra @ 9:46 AM

4 @ 10:35 AM / 4.32

0.4% PFA @ 10:48

8 AM
Special

+ 4 extras
Time 14 hrs

Done

Spin @ 8:12

400pl Zombie @ 8:28 → 8:43 spin @ 8:44 AM

Hot @ 9:08 am → 9:38 spin @ 9:38 AM

Cold @ 9:52 → 10:22 AM spin @ 10:22 AM

Strept @ 10:39 am → 10:54 spin @ 10:54 AM

Fix 11:06 → 7:16 → 26 → 36

1 @ 11:39 am

2 @ 11:48 am

Intra @ 12:00 pm

3 @ 12:43 pm

4 @ 12:51

0.4% PFA / 4.39

Sc's spin @ 7:35 AM

Cold @ 7:55 AM → 8:25

Hot @ 7:59 AM → 8:29

Both spin @ 8:32 AM

Merged w

Time point 12 hrs

for fix steps

Bulk Done @ 9:39 am

All NY031 got regular

372 due left over

40 + 20 residue 372

Note case for 4% crash

Accident w/ Icos

@ Time 14 hrs all

got 10 ph old

Icos down vs 3 ph.

For the 4 Icos test NY031

0.5, 0.8, 1.0 & 1.2

Waiting @ 8:33
for the Sc's
to get wash.

Note Viability ≠ for 4 test NY031
~ 3/4 drops each expect
Δ from samples

412

3.2

December 29th, 2024

αβ T cell SFC panel																	
#	Filter	Fluorochrome	Marker	Clone	Dose	During stim!!!	33	L/D RT 15 min	RT for 10 min	Hot Stain 30min @4C	16	Cold Stain 30min @4C	16	4°C for 15 min	Rec Lysate then perform	Intracellular Stain 40 min @RT	16
1	UV1	BUV395	CD62L	(DREG-56)	1							<1.2	#####				
2	UV6	BUV563	CD69	(FN50)	2												
3	UV9	BUV615	CD69	(FN50)	2												
4	UV10	BUV650	CD8	(J252D4)	1												
5	UV11	BUV661	CD8	(J252D4)	1												
6	UV13	BUV711	CD8	(J252D4)	1												
7	UV14	BUV737	CD38	(B6)	2												
8	UV16	BUV805	CD4	(SK3)	1												
9	V1																
10	V2																
11	V3	Pacific Blue	CD14	(M5E2)	2												
12	V4	Pacific Blue	CD19	(H1B19)	2												
13	V5	BUV480	CD161	(REA631)	1												
14	V6																
15	V7																
16	V8	BUV570	CD16		1												
17	V9	BUV605	CD45RA	(J252D4)	1												
18	V10	BUV650	CD8		1												
19	V11	BUV711	CD8		1												
20	V13	BUV750	CD8		2												
21	V14	BUV750	CD8		2												
22	V15	BUV786	CD8	(11A9)	1												
23	V16	Alexa Fluor 488	CD8		1												
24	B2	Alexa Fluor 488	CD8		1												
25	B3	Spark Blue 550	CD3	(SK7)	1												
26	B4	Spark Blue 574	CD45		1												
27	B6	RB613	PD1		1												
28	B7																
29	B8																
30	B9																
31	B10	RB705	CD26	(M-A261)	1												
32	B12																
33	B13																
34	B14	RB780	CD85?		2												
35	YG1	PE	ICOS	(EH12.2H7)	1												
36	YG2	PE-Dazzle 594	TNFR	(MAB11)	2												
37	YG3																
38	YG4	PE-Cy5	CD83		2												
39	YG5																
40	YG6	PE-Fire 700	CD127		1												
41	YG7	PE-Fire 744	CD25		2												
42	YG8	PE-Vio 770	HLA-DR	(L243)	1												
43	YG9	PE-Fire810	CD39	(A1)	1												
44	R1	APC															
45	R2	Alexa Fluor 647	IL2	(MOL-2)	1												
46	R3																
47	R4	APC-R700	CD107a	(H4A3)	0.6	19.8											
48	R5																
49	R6	Zombie NIR	L/D		2												
50	R7	APC-Fire 750	CD27	(O323)	1												
51	R8	APC-Fire 810	CD87		1												
And UNSTAINED CONTROLS !!!																	
						0.6	19.9	Antibody Total	6.23	99.68	16	7.3	116.8	4.9	78.4		
						19.5	15.69	Brilliant Stain	12.46	199.36	14.6	233.6	9.8	156.8			
								Pipette draw volume	15.69	18.9				11.7			

αβ T cell SFC Panel

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 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 PermWash: 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 PermWash: 1.5 ml NuclearWash 1500 rpm 6 min
Resuspend in 80 ul 0.4% PFA-FBS
Cap tubes, wrap rack in foil, store at 4°C

Facilities, Howard Hall 1324

14 L N4068
10 E6E ICOS vs 3p1

10pL

12pL

8pL

ICOS 3pL
Strep 29.5pL
ICOSTest 20pL (81.5)
0.5 0.8 1.0 1.2
25
x.4
10.0

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Rationale = Not wasting antibodies

~ Abs ~ $\mu\text{L} \rightarrow \frac{\mu\text{g}}{\text{mL}} \rightarrow \mu\text{g add}$

$\mu\text{g add}$ per tube volume

* time * temp

* antigen etc.

$\frac{1}{2}$ the μg added.

If already saturated, no effect.

If borderline, then ~50% drop?

somewhere in between
both outcomes.

21 SCS + 24 samples

$$\begin{array}{r} 21 \\ + 24 \\ \hline 45 \\ \times .3 \\ \hline \boxed{13.5} \end{array} \quad \begin{array}{r} 24 \\ \times .6 \\ \hline 14.4 \end{array} \quad \begin{array}{r} 12.6 \\ \times 2 \\ \hline 25.2 \end{array}$$

$$\begin{array}{r} 12.6 \\ \times 8 \\ \hline 100.8 \end{array}$$

$$\begin{array}{r} 1.7 \leftarrow \\ 1.5 + 1.5 \leftarrow 3.0 \end{array}$$

$$1.4 \times 4 = 5.6$$

$$12.0$$

$$\boxed{22.3.}$$

$$\begin{array}{r} 22.3 \rightarrow 44.6 \\ \times 5 \\ \hline \boxed{223.0} \end{array}$$

$$\begin{array}{r} 12 \cdot 12 \cdot 12 \\ \times 19 \\ \hline 108 \\ 120 \\ \hline \boxed{128} \end{array}$$

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