

December 29th, 2022

CD161 and hMRI

2 LHM LHM

Specimen	Status	Location	Conc	Date	Tasks	Volume	Ly	Ly+Mon	Total	2E+6	3E+6
DOC6	Adult	11/12/2021	15E+6			4	3.31E6	4.32E6	13.24 (17.28)	.462	
						5	4.13E6	5.48E6	20.65 (27.4)	.365	
									34 million (44.7)		

Allied Health closed ...

5:48 am prep

Stain count @ 6:42 am → 6:49

Count @ 6:54 am

Incubator @ 7:44 am → 1:44 pm

@ 1/1 CH + 500  $\mu$ l (total)

~ 3.0 E/L

... CD107a?

@ 8:07 am (sigli)

11:52 am Abs prepped  
A 10% FBS

11:41 pm

2:30 pm 1/2 in

→ 2:45 pm 40 mm

500 CD107a + Biotin sds in  
3:40 spin @ 5:43 pm

5:13 for 161 tets

5:15

new OP-RU

@ 5  $\mu$ l

5:22 for reg tets

→ 5:02

will get 2  $\mu$ l CD161 hot

SC's @ 15:32 → 16:02 pm

2/3 SC unstained → new PDI

Tetramer sds @ 3:50 → 4:29?

Sds RBC lysis @ 6:03

Tets & Sds spin 1300/20 min @ 10:09  
(161 tets in RT for 10 min while wait buddy)

29.47 ~ 1000  
1000 → 34 m

CD161 RF  
minutes before not

2  $\mu$ l 11000

20  
x .8  
16.00

~ 7  $\mu$ l in 17.5 mls

20  
x .08  
1.6 mls  
PEA-PBS

30  
x .3  
9 mls  
RBC  
lyse

120  
x 3  
360

20 (unst)  
x 8  
160 mls  
20 mls

180 mls FBS-PBS

20  
x 4  
80 mls Permablush  
+ 20  
100 mls Permablush

	CD161e Tet	CD161 hot	CD161 cold	CD161 direct
Normal	1.5 $\mu$ m			
Spiked				
	10:10	5:10	3:10	Biotin + Tet
hMRI OP-RU (new)				
6-PP				
hMRI OP-RU old				Biotin + Tet
6-PP				
				CD107A
				CD107A
				CD45RA
				CD3
				CD16
				CD161
				CD161

18  
x 3  
54 million cells [3.43]

New vials of both hMRIs, spin down in clear vial, take from top. PDI

19  
x 2  
38  
3.6  
41

2.0 - 4.32  
~ 1.5 3.31

CD107A also impacts ~

11 635  
x 538 x 15

5,918 [9,525]

11 15  
x 38 x 133  
418  $\mu$ l 2,025

4:29 pm Tet sc's done (FBS wash), spin @ 4:30 pm 1400/6

4:23 pm → 33 pm <sup>10 min</sup> CD161 head start on the tet experiment <sup>+ hot stain</sup> (CD161 not get's 2 pl next)

Sc's Fix Perm @ 4:26 → 36 → 46 pm

Samples @ 16:39 for 37°C → 17:09 pm

tet sc's @ 16:41 pm

10x lysis

+ sc's 1st perm wash → 16:48 pm

For Perm wash @ 4:57 pm → 07:17 pm

Sc's w/ 0.4% PEA @ 5:10 pm  
2nd perm wash for sc's @ 5:00 pm ...

37°C wash samples @ 5:16 pm ✓

Spilled sc's @ 5:18 → 5:58 pm

4°C samples @ 5:30 pm → 6:00 pm

4 ~~37°C~~ spin post RBC lysis + lost 2 sc's @ 6:09 pm

Samples Fix Perm @ 6:22 pm → 32 → 42

Sc's done @ 6:26 pm

6:58 pm

actual samples 10 f perm wash

Sc's "extra totally intentional perm wash" → 7:00 pm

2nd Perm wash @ 7:04 pm

1 Va 2.2<sup>+</sup> added to single tube @ 7:22 ... Upps! (instead of @ cold step it's now all @ later ...)

Intraacellular @ 7:26 pm → 8:06 pm

3rd wash @ 8:02 pm

Done @ 8:26 pm → 13:40:00 <sup>stem stain count //</sup>

x4 perm washes  
maybe 5 for FDI  
a core



#	Filter	Single color (ul)	Ref ctrl	Limiting ctrl name	Fluorescence	Marker	Clone	Val/lot #	During stim II	20	L/D 15 min (RT)	Tetramer 40 min @ RT	HisStain 30min @ 37C	20	Coldestain 30min @ 4C	20	RBC Lys, then FcyPerm	Intracellular Stain 40 min @ RT	20
1	UV2				AF	CD22	(ORIG-35)								1.2	24.0			
2	UV7				AF-UV6	CD22	(ORIG-35)												
3	UV9				BUV496	CD8	(BP2-13)								0.7	14.0			
4	UV10				BUV463	CD9	(7H50)								0.5	10.0		1	20.0
5	UV11				BUV435	CD4	(131)												
6	UV14				BUV61	CD2	(86)								0.7	14.0			
7	UV16				BUV37	CD3	(1C6)								2.5	50.0			
8	UV1				BUV805	CD4	(SK3)												
9	UV3				BUV421	CD127	(4012925)												
10	UV5				Pacific Blue	CD14	(M5E2)								2.0	40.0			
11	UV7				Pacific Blue	CD19	(H1B13)								2.0	40.0			
12	UV10				BUV480	CD161	(B6A33)								<2>	14.0			
13	UV11				BUV655	CD36									0.7				
14	UV14				BUV711	CD7													
15	UV15				BUV750	CD8	(B27)												
16	UV16				BUV755	CD8	(11A9)												
17	UV17				Allophycocyanin 488	CD3	(SK3)												
18	UV18				PE	CD3													
19	UV19				PE-CF554	CD3	(M-A351)												
20	UV20				PE-CF554	CD3	(M-A351)												
21	UV21				PE-CF554	CD3	(M-A351)												
22	UV22				PE-CF554	CD3	(M-A351)												
23	UV23				APC	CD16													
24	UV24				APC	CD16													
25	UV25				APC	CD16													
26	UV26				APC	CD16													
27	UV27				APC	CD16													
28	UV28				APC	CD16													
29	UV29				APC	CD16													
30	UV30				APC	CD16													
31	UV31				APC	CD16													
32	UV32				APC	CD16													
33	UV33				APC	CD16													
34	UV34				APC	CD16													
35	UV35				APC	CD16													
36	UV36				APC	CD16													
37	UV37				APC	CD16													
38	UV38				APC	CD16													
39	UV39				APC	CD16													
40	UV40				APC	CD16													
41	UV41				APC	CD16													
42	UV42				APC	CD16													
43	UV43				APC	CD16													
44	UV44				APC	CD16													
45	UV45				APC	CD16													
46	UV46				APC	CD16													
47	UV47				APC	CD16													
48	UV48				APC	CD16													
49	UV49				APC	CD16													
50	UV50				APC	CD16													
51	UV51				APC	CD16													
52	UV52				APC	CD16													
53	UV53				APC	CD16													
54	UV54				APC	CD16													
55	UV55				APC	CD16													
56	UV56				APC	CD16													
57	UV57				APC	CD16													
58	UV58				APC	CD16													
59	UV59				APC	CD16													
60	UV60				APC	CD16													
61	UV61				APC	CD16													
62	UV62				APC	CD16													
63	UV63				APC	CD16													
64	UV64				APC	CD16													
65	UV65				APC	CD16													
66	UV66				APC	CD16													
67	UV67				APC	CD16													
68	UV68				APC	CD16													
69	UV69				APC	CD16													
70	UV70				APC	CD16													
71	UV71				APC	CD16													
72	UV72				APC	CD16													
73	UV73				APC	CD16													
74	UV74				APC	CD16													
75	UV75				APC	CD16													
76	UV76				APC	CD16													
77	UV77				APC	CD16													
78	UV78				APC	CD16													
79	UV79				APC	CD16													
80	UV80				APC	CD16													
81	UV81				APC	CD16													
82	UV82				APC	CD16													
83	UV83				APC	CD16													
84	UV84				APC	CD16													
85	UV85				APC	CD16													
86	UV86				APC	CD16													
87	UV87				APC	CD16													
88	UV88				APC	CD16													
89	UV89				APC	CD16													
90	UV90				APC	CD16													
91	UV91				APC	CD16													
92	UV92				APC	CD16													
93	UV93				APC	CD16													
94	UV94				APC	CD16													
95	UV95				APC	CD16													
96	UV96				APC	CD16													
97	UV97				APC	CD16													
98	UV98				APC	CD16													
99	UV99				APC	CD16													
100	UV100				APC	CD16													

**Simplified Protocol**

Thaw cells, DNase, count

Collect, count, aliquot cells 3.0E-6 cells R10 5ml polypropylene tube

Bring volume up to 1 ml R10, add 2 ul PMAActiv and DuringStim antibody

Cap and incubate at 37°C for 6 hours

Wash with 2 ml PBS, spin down 1300rpm 8min

800 ul of Livebead mix (1:2500) @RT for 15min

Wash 2 ml 5% PBS-FBS, spin 1300 rpm, 8min

Add tetramers for 40 minutes at RT

Wash with 2 ml PBS, spin down 1300rpm 8min

Add HisStain mix, incubate @37°C for 30 min

Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

Add Coldestain mix, incubate @ 4°C for 30min

Add 300-500 ul 1x RBC Lysis for 3 minutes

Wash 2 ml 5% PBS-FBS 1400 rpm, 6min

300 ul BD FcyPerm, incubate @ 4°C for 20min

(vortex every 10 minutes)

First PermWash: 1 ml PermWash 1500 rpm 6 min

Second Perm Wash: 1 ml PermWash 1500 rpm 6 min

Add Intracellular Stain, incubate @ RT for 40min

First PermWash: 1 ml PermWash 1500 rpm 6 min

Second Perm Wash: 1 ml PermWash 1500 rpm 6 min

Resuspend in 100 ul 0.4% PFA-PBS

Cap tubes, wrap rack in foil, store at 4°C

New PDI  
New col107a  
Abc Pappad @ 11:36am

c Spillages col161 ~ 10:55  
PDI >

1.2 for  
V. 1.2 for

1955  
1.5.20

13.14.11  
1.2.20

December 29th, 2022

CD161 and hMR1

4:03 pm start

654,000 counts 10/10

50's 50'' ~ 40

100k ~ 30

d'Is looking cold & kinetic temp  
or a down modulation?



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CD161 and hMR1

