Feb 09, 2021

Immunofluorescent methods for antibody test in mouse colon

Lixin Wang[1], Pu-Qing Yuan[1], Honghui Liang[1], Yvette Tache[1]

[1]University of California, Los Angeles

1 Works for me dx.doi.org/10.17504/protocols.io.bqi2muge

SPARC
Tech. support email: info@neuinfo.org

Manmeet Bains

SUBMIT TO PLOS ONE

ABSTRACT

A table listed the information of antibodies used in the mouse colon. The protocol described the methods of colon tissues sampling, whole mount preparations, passive clarity technique, and respective fluorescent Immunohistochemistry for the antibody tests in different tissue preparations.

DOI

dx.doi.org/10.17504/protocols.io.bqi2muge

PROTOCOL CITATION

Lixin Wang, Pu-Qing Yuan, Honghui Liang, Yvette Tache 2021. Immunofluorescent methods for antibody test in mouse colon. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bqi2muge

KEYWORDS

antibody, Immunofluorescence, enteric nervous system, colon, mouse

CREATED

Dec 08, 2020

LAST MODIFIED

Feb 09, 2021

PROTOCOL INTEGER ID

45370

MATERIALS TEXT

a. *Animals:* Mice C57BL/6J (Jackson Laboratories, Sacramento, CA), male and female, age range of 6-10 weeks old.
b. isoflurane
c. oxygen
d. induction chamber
e. Sylgard™ 184 silicone elastomer (Electron Microscopy Science, Hatfield, PA)
f. saline
g. 0.01 M phosphate buffered saline
h. primary and fluorescent protein-conjugated 2ndantibodies
i. Refractive Index Matching Solution:

Histodenz (Sigma D2158)	40 g	88%
Tween-20	30 µl	0.1%
Sodium azide	3 mg	0.01%
0.02 M phosphate buffer (pH7.5)	30 ml	

j. microscopic glass slides and cover slides

Tissue Collection

1 Mice were euthanized by an overdose of 5% isoflurane in oxygen.

2 After thoracotomy, perfusion cannula (18G) was inserted into the aorta via the left ventricle.

3 Saline was perfused for ~1 min to flush out the blood.

4 The colon was removed from ileocecal junction to the end of distal colon at the level of pelvic brim where runs the iliac artery, and flat-pinned on a Sylgard™ 184 silicone elastomer (Electron Microscopy Science, Hatfield, PA).

5 The colon was fixed in 4% paraformaldehyde at 4°C overnight.

6 The tissues were rinsed in 0.01 M phosphate-buffer saline (PBS).

Whole Mount Preparation

7 Whole mounts of the submucosal and myenteric plexuses of mouse proximal and distal colon were prepared under a stereomicroscope:

Scrape off the mucosa using an angled fine tip forceps

8 Peel off the submucosa layer containing the submucosal plexus using a micro-scissors.

9 Remove the circular muscles carefully using fine forceps

Citation: Lixin Wang, Pu-Qing Yuan, Honghui Liang, Yvette Tache (02/09/2021). Immunofluorescent methods for antibody test in mouse colon. https://dx.doi.org/10.17504/protocols.io.bqi2muge

10 The remaining layers of the whole mount preparation includes the myenteric plexus adhered on the longitudinal muscle and serosa.

Some samples had the circular muscles remained, i.e. the layers include the circular and longitudinal muscles with the myenteric plexus in between, and serosa, which are indicated in the antibody table.

PACT (passive clarity technique)

11 Rinse 3 times the colon samples remained flat-pinned on the Sylgard silicone dish in PBS with 0.02% NaN3

12 Immerse the colon wall in Hydrogel A4P0 (4% acrylamide without paraformaldehyde) solution for 1 day at 4°C

13 Place the colon in 50 ml tube with 5-10 ml hydrogel

14 De-gas for 10 min in a desiccation chamber filled with nitrogen with a vacuum pump on (http://wiki.claritytechniques.org/index.php/CLARITY_Technique)

15 Polymerize in 37°C water bath 1 h, then remove the extra gel as much as possible

16 Rinse by 8% SDS overnight and then 4% SDS for 2 days in 37°C shaker

17 Rinse 3 times in PBST (0.1% Triton-X in 1X PBS with 0.02% NaN3) at 37°C on a shaker plate 1 day, store in PBS-NaN3

Fluorescent Immunohistochemistry

18 **For whole mount preparation and transverse sections:**

10% NDS in 0.3% Triton-X 100-PBS 1 h at room temperature (RT)

19 1st antibody in 0.3% Triton-X 100-PBS 2 h RT, 4°C 2 days

20 Rinse in 1X PBS 30' x 5 times

21 2nd antibody RT 3 h

Citation: Lixin Wang, Pu-Qing Yuan, Honghui Liang, Yvette Tache (02/09/2021). Immunofluorescent methods for antibody test in mouse colon. https://dx.doi.org/10.17504/protocols.io.bqi2muge

22 Rinse 1X PBS 5 times

23 Mount and coverslip in Vectashield (Vector Laboratories, Burlingame CA)

24 **For FACT-cleared colon wall:**

10% NDS in 0.3% Triton-X 100-PBS 1 h RTat room RT, and then 4°C overnight

25 1st antibody in 0.3% Triton-X 100-PBS 3 h RT, 4°C 3-5 days

26 Rinse in 1X PBS 1 h x 5 times, then 4°C overnight

27 2nd antibody in 0.3% Triton-X 100-PBS 3 h RT, 4°C 1-2 days

28 Rinse in 1X PBS 1 h x 5 times, then 4°C overnight

29 Immersed in a custom-made imaging media, RIMS (ref) with a reflective index of 1.46], 2 h at RT and overnight at 4°C.

30 Embedded on microscopic glass slides in fresh RIMS sealed by iSpacer (SunJin Lab Co., Hsinchun City, Taiwan, www.sunjinlab.com). The colon samples were placed onto the slides with serosa on top.

Citation: Lixin Wang, Pu-Qing Yuan, Honghui Liang, Yvette Tache (02/09/2021). Immunofluorescent methods for antibody test in mouse colon. https://dx.doi.org/10.17504/protocols.io.bqi2muge