Sep 21, 2022

# Preparation and Immunohistochemistry of Mouse Small Intestine and Colon

Nathan D Rossen[1]

[1]UCSF



dx.doi.org/10.17504/protocols.io.j8nlkw575l5r/v1

SPARC
Tech. support email: info@neuinfo.org

Nathan D Rossen

ABSTRACT

Mice were euthanized, the small and large intestine collected, and fixed frozen sections prepared. The microscope mounted tissue sections were stained using immunohistochemistry. Expression of specific proteins and reporter proteins were visualized using microscopy.

DOI

dx.doi.org/10.17504/protocols.io.j8nlkw575l5r/v1

PROTOCOL CITATION

Nathan D Rossen 2022. Preparation and Immunohistochemistry of Mouse Small Intestine and Colon. **protocols.io** https://protocols.io/view/preparation-and-immunohistochemistry-of-mouse-smal-cguhtwt6



CREATED

Sep 19, 2022

LAST MODIFIED

Sep 21, 2022

PROTOCOL INTEGER ID

70249

MATERIALS TEXT

**Primary Antibodies**

| A | B | C | D | E | F |
|---|---|---|---|---|---|
| Target | Host | Dilution | Manufacturer | Catalog number | RRID |
| Serotonin | Rabbit | 1:5000 | ImmunoStar | Cat# 20080 | RRID:AB_572263 |
| Serotonin | Goat | 1:500 | Abcam | Cat# ab66047 | RRID:AB_1142794 |
| GFP | Chicken | 1:500 | Aves Labs | Cat# GFP-1010 | RRID:AB_2307313 |
| dsRed | Rabbit | 1:500 | Takara Bio | Cat# 632496 | RRID:AB_10013483 |

**Secondary Antibodies**

| A | B | C | D | E | F |
|---|---|---|---|---|---|
| Conjugate/Target | Host | Dilution | Manufacturer | Catalog number | RRID |
| AF647 anti-rabbit | Donkey | 1:500 | Invitrogen | Catalog # A32795 | RRID:AB_2536183 |
| AF647 anti-goat | Donkey | 1:500 | Invitrogen | Catalog # A-21447 | RRID:AB_141844 |
| AF488 anti-chicken | Donkey | 1:500 | Invitrogen | Catalog # A78948 | RRID:AB_2921070 |
| AF564 anti-rabbit | Donkey | 1:500 | Invitrogen | Catalog # A10042 | RRID:AB_2534017 |

**Other Reagents**

OCT (Optimal Cutting Temperature compound) **Sakura Finetek Catalog #4583**

Donkey Serum **Emd Millipore Catalog #S30-100ML**

PBS - Phosphate-Buffered Saline (10X) pH 7.4 **Thermo Fisher Scientific Catalog #AM9625**

Triton X-100 **Sigma Aldrich Catalog #T8787-50ML**

Bovine Serum Albumin **Sigma Aldrich Catalog #A7030**

Sucrose **Sigma Aldrich Catalog #S7903**



**Citation:** Nathan D Rossen Preparation and Immunohistochemistry of Mouse Small Intestine and Colon https://dx.doi.org/10.17504/protocols.io.j8nlkw575l5r/v1

Prolong Gold **Thermo Fisher Scientific Catalog #P36930**

Paraformaldehyde 32% (methanol free) **Electron Microscopy Sciences Catalog #15714**

**Composition of Abbreviated Solutions**

PBS: phosphate-buffered saline, 0.1 M, pH 7,2

Blocking buffer: 5% donkey serum, 5% BSA, .3% Triton X-100 in PBS

## Tissue Collection and Preparation

1 Mice 6 - 8 weeks old were euthanized by isolfurane administration followed by cervical dislocation.

2 Mice were transcardially perfused with 5ml ice cold PBS.

3 Whole small intestine and colon were collected and kept in PBS on ice. The small intestine was dissected into three segments of approximately equal length.

4 Each segment (proximal, medial, and distal small intestine and whole colon) was flushed with 5ml ice cold PBS, cut lengthwise, and laid flat with the mucosal surface facing up. Each segment was further washed with ice cold PBS.

5 Tissue segments were washed with ice cold 4% PFA in PBS, and then laid flat in between two layers of filter paper and fixed in 4% PFA in PBS at 4C for either 4hrs or 24hrs.

6 Tissue was rinsed in excess PBS and transferred to 30% sucrose solution for at least 16 hours.

7 Tissue was transferred to a mixture of 50% OCT, 15% sucrose in PBS and rolled on a wooden stick.

8 Tissue was frozen in OCT as a "swiss roll" and sectioned into 5um or 20um sections on a cryostat and kept at -80C.



**Citation:** Nathan D Rossen Preparation and Immunohistochemistry of Mouse Small Intestine and Colon https://dx.doi.org/10.17504/protocols.io.j8nlkw575l5r/v1

## Immunohistochemistry

9 Slide-mounted tissue sections were thawed and air dried for 30 minutes

10 Sections were washed twice for 10 minutes in PBS and once for 10 minutes in .2% Triton X-100 in PBS.

11 Sections were blocked for 1 hour in blocking buffer at room temperature.

12 Sections were incubated with primary antibodies in blocking buffer overnight at 4C.

13 Sections were washed thrice for 10 minutes in .2% Triton X-100 in PBS.

14 Sections were incubated with secondary antibodies and DAPI in blocking buffer for 1-2 hours at room temperature.

15 Sections were washed twice for 10 minutes in .2% Triton X-100 in PBS and twice for 10 minutes in PBS.

16 Sections were mounted in Prolong Gold Mounting Media.



**Citation:** Nathan D Rossen Preparation and Immunohistochemistry of Mouse Small Intestine and Colon
https://dx.doi.org/10.17504/protocols.io.j8nlkw575l5r/v1