

JUL 16, 2023

• Immunofluorescence staining of myenteric and submucosal plexuses

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ABSTRACT

This protocol details myenteric and submucosal plexus immunostaining.

ATTACHMENTS

786-2005.pdf

MATERIALS

Materials

Normal Donkey Serum Jackson ImmunoResearch Laboratories, Inc. Catalog #017-000-121

Rabbit anti-tyrosine hydroxylase antibody; AB_390204 Merck Millipore (EMD Millipore) Catalog #AB152

CD3 antibody | 145-2C11 Bio-Rad Laboratories Catalog #MCA2690

X IBA Ab Wako Catalog #019-19741

Recombinant Anti-CD68 antibody [FA-11] **Abcam Catalog** #ab53444

- 1% Triton-X
- PBS
- DAPI

OPEN BACCESS

DOI:

dx.doi.org/10.17504/protocol s.io.q26g7p85kgwz/v1

Protocol Citation: Connor Monahan 2023. Immunofluorescence staining of myenteric and submucosal plexuses. **protocols.io**

https://dx.doi.org/10.17504/protocols.io.q26g7p85kgwz/v1

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Protocol status: Working We use this protocol and it's working

Created: |ul 12, 2023

Last Modified: Jul 16, 2023

PROTOCOL integer ID: 84907

Procedure

5h

1

Note

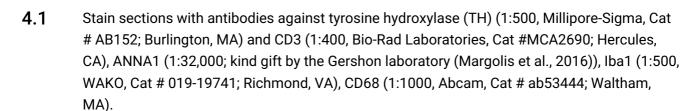
Note: The plexuses are fragile. At each wash, use a micropipette and a dissecting microscope to carefully remove the solution from the well not suck up the plexus.

Cut out two small regions of each plexus sheet in PBS at [4 ° C].

- 2 Add each tissue section to separate wells in a 96 well dish.
- Add 10% normal donkey serum (Jackson Immunoresearch, Cat #017-000-121; West Grove, PA)

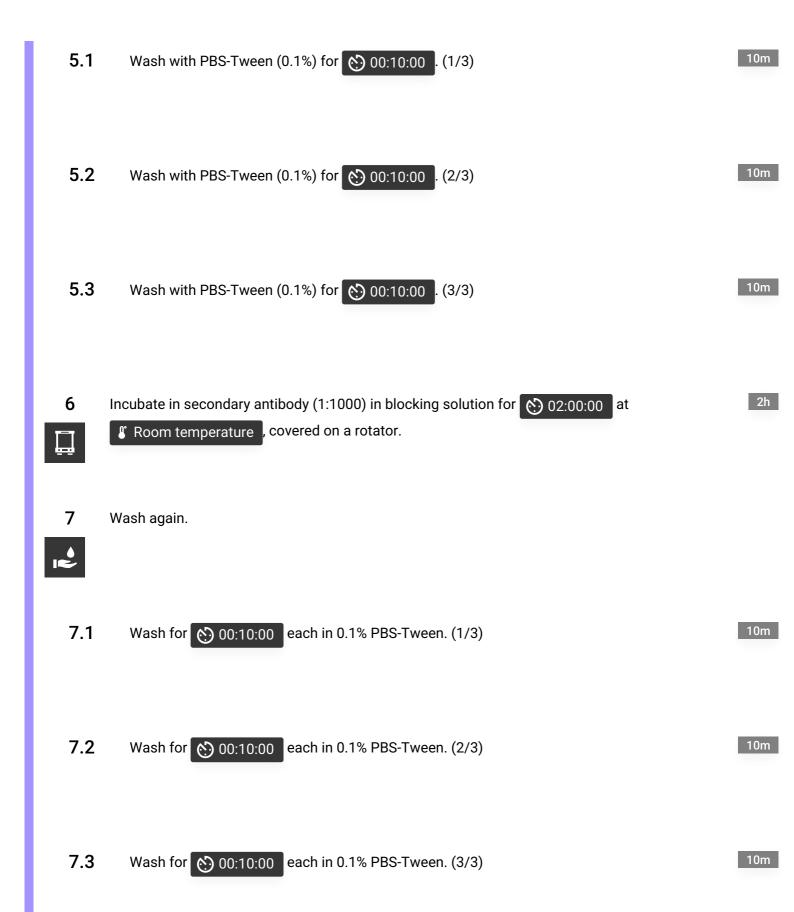


- and 1% Triton-X in PBS. Leave in blocking solution for 01:00:00 at Room temperature on a rotator.
- Incubate the tissue Overnight at Room temperature on a rotator with primary antibodies in 10% normal donkey serum, 1% Triton-X in PBS.



5 On Day 2, remove the primary antibody solution from each well and wash.





8 Mount on slide with vectashield medium with DAPI

9 Imaging:



- 9.1 For imaging enteric neurons, for each plexus collect 2-3, 2x2 tile z-stack $640.17x640.17 \mu m$ confocal images at 20x magnification.
- **9.2** For imaging macrophages, for each plexus collect 2-3, 2x2 tile z-stack 390.09 x 390.09 μm confocal images at 20x magnification.
- 10 Analysis:



- 10.1 Count the number of ANNA1⁺, TH⁺ cells, and IBA1⁺ cells for each stacked image using Fiji.
- Within the SP, threshold the TH⁺ signal, then analyze mean fluorescent intensity (MFI) and the area of the TH signal. Keep the thresholding consistent across each image, animal, and condition within each experiment.
- 10.3 Within each animal, sum the the number of ANNA1⁺, TH⁺ cells, and IBA1⁺ cells across all images separately then divide by the acquisition area.
- **10.4** For each experiment, normalize to the CFA only condition.