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We use this protocol and it's working

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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**

IBA Ab **Wako Catalog #019-19741**

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

- 1% Triton-X
- PBS
- DAPI

Keywords: myenteric plexus, submucosal plexus, immunostain, immunofluorescence, enteric neuron, ileum, macrophage, T cell

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.

3 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. 1h

4 Incubate the tissue Overnight at Room temperature on a rotator with primary antibodies in 10% normal donkey serum, 1% Triton-X in PBS. 1h

4.1 Stain sections with antibodies against tyrosine hydroxylase (TH) (1:500, Millipore-Sigma, Cat # AB152; Burlington, MA) and CD3 (1:400, Bio-Rad Laboratories, Cat #MCA2690; Hercules, CA), ANNA1 (1:32,000; kind gift by the Gershon laboratory (Margolis et al., 2016)), Iba1 (1:500, WAKO, Cat # 019-19741; Richmond, VA), CD68 (1:1000, Abcam, Cat # ab53444; Waltham, MA).

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

5.1 Wash with PBS-Tween (0.1%) for 00:10:00 . (1/3) 10m

5.2 Wash with PBS-Tween (0.1%) for 00:10:00 . (2/3) 10m

5.3 Wash with PBS-Tween (0.1%) for 00:10:00 . (3/3) 10m

6 Incubate in secondary antibody (1:1000) in blocking solution for 02:00:00 at Room temperature , covered on a rotator. 2h

7 Wash again.

7.1 Wash for 00:10:00 each in 0.1% PBS-Tween. (1/3) 10m

7.2 Wash for 00:10:00 each in 0.1% PBS-Tween. (2/3) 10m

7.3 Wash for 00:10:00 each in 0.1% PBS-Tween. (3/3) 10m

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 µm 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.

10.2 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.