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Immunofluorescence staining of myenteric and submucosal plexuses

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details myenteric and submucosal plexus immunostaining.

Attachments



[786-2005.pdf](#)
53KB

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



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, 2×2 tile z-stack 640.17×640.17 μm confocal images at 20x magnification.
- 9.2 For imaging macrophages, for each plexus collect 2-3, 2×2 tile z-stack 390.09 × 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.