SPARC Serotonin (5-HT) Immunohistochemistry Protocol in Rat Tissues Labeled with Cholera Toxin B-fragment

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1 Works for me dx.doi.org/10.17504/protocols.io.2kggctw

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ABSTRACT

This protocol describes the immunofluorescent labeling technique used to identify serotonin expression in CtB-labelled phrenic motor neurons and within a defined region of interest surrounding phrenic motor neurons.

1 Day 1: primary antibodies required:
5-HT: Rabbit anti-5-HT (Immunostar #20080)
Cholera toxin B-fragment: Goat anti-CT-B (Millipore #227040)

2 Place 40um transverse spinal cord sections into 1xPBS-Triton (0.1%) in 12 well cell culture plates

3 5x washes in 1xPBS-Triton (0.1%) for 5 minutes each at room temperature

4 Antigen retrieval: place tissues into Heat-Induced Epitope Retrieval (TissuePro, Cat#: HIER01-32R) for 30 minutes at 85 degrees C

5 5x washes in 1xPBS-Triton (0.1%) for 5 minutes each at room temperature

6 Blocking: place tissues in 5% Normal Donkey Serum (NDS) in 1xPBS-Triton (0.1%) for 60 minutes at room temperature

7 Primary Antibody Incubation: Incubate tissues in: 5%NDS in 1xPBS-Triton (0.1%), Rabbit anti-5-HT (1:2000), and Goat anti-Ct-B (1:2500) for 1 hour at room temperature

8 Continue primary antibody incubation overnight at 4 degrees C

9 Day 2: secondary antibodies required:
AlexaFluor 488: donkey anti-goat (Invitrogen, Ref#A11055)
AlexaFluor 594: donkey anti-rabbit (Invitrogen, Ref#A21207)

10 5x washes in 1xPBS-Triton (0.1%) for 5 minutes each at room temperature

11 Secondary Antibody Incubation: Incubate tissues in: 5%NDS in 1xPBS-Triton (0.1%), donkey anti-goat (1:1000), and donkey anti-rabbit

(1:500) for 2 hours at room temperature

12 5x washes in 1xPBS for 5 minutes each at room temperature

13 Mount tissues on Superfrost Plus microscope slides

14 Allow slides to dry overnight

15 Coverslip with VectaShield Antifade Hard Set Mounting Medium (Cat#:H-1400)