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

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Protocol status: Working

We use this protocol and it's working

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Keywords: serotonin, 5HT, 5-HT, immunofluorescence, phrenic, immunohistochemistry, CT-B, cholera toxin B fragment, serotonin expression in ctb, sparc serotonin, serotonin expression, labelled phrenic motor neuron, phrenic motor neuron, surrounding phrenic motor neuron, immunohistochemistry protocol in rat tissue, immunofluorescent labeling technique, immunohistochemistry protocol, rat tissue

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.

Troubleshooting

- 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)