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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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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)