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SPARC Serotonin 2A Receptor (5-HT2AR) Immunohistochemistry Protocol in Rat Tissues Labeled with Cholera Toxin B-fragment

In 1 collection

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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 2A receptor 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-HT2AR: Rabbit anti-5-HT2AR (Immunostar #24288)
Cholera toxin B-fragment: Goat anti-CT-B (Millipore #227040)

2 Place 40um transverse spinal cord sections into 1xPBS in 12 well cell culture plates

3 5x washes in 1xPBS for 5 minutes each at room temperature

4 Blocking: place tissues in 5% Normal Horse Serum (NHS) in 1xPBS-Triton (0.1%) +0.5% bovine serum albumin (BSA) for 60 minutes at room temperature

5 Primary Antibody Incubation: Incubate tissues in: 5%NHS in 1xPBS-Triton (0.1%), Rabbit anti-5-HT2AR (1:1000), and Goat anti-Ct-B (1:2500) for 48 hours at 4 degrees C

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

7 5x washes in 1xPBS for 5 minutes each at room temperature

8 Secondary Antibody Incubation: Incubate tissues in: 5%NHS in 1xPBS-Triton (0.1%), donkey anti-goat (1:1000), and donkey anti-rabbit (1:1000) for 2 hours at room temperature

9 5x washes in 1xPBS for 5 minutes each at room temperature

10 Mount tissues on Superfrost Plus microscope slides

11 Allow slides to dry overnight

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