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# SPARC Serotonin 7 Receptor (5-HT7) 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 7 receptor 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-HT7 Receptor: Rabbit anti-5-HT7R (Immunostar #244430)  
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 3x washes in 1xPBS 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 3x washes in 1xPBS for 5 minutes each at room temperature
- 6 Blocking: place tissues in 10% 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-5HT7R (1:2000), and Goat anti-Ct-B (1:2500) overnight at 4 degrees C
- 8 Day 2: secondary antibodies required:  
AlexaFluor 488: donkey anti-goat (Invitrogen, Ref#A11055)  
AlexaFluor 594: donkey anti-rabbit (Invitrogen, Ref#A21207)
- 9 3x washes in 1xPBS for 5 minutes each at room temperature
- 10 Secondary Antibody Incubation: Incubate tissues in: 5%NDS in 1xPBS-Triton (0.1%), donkey anti-goat (1:1000), and donkey anti-rabbit (1:1000) for 2 hours at room temperature
- 11 3x washes in 1xPBS for 5 minutes each at room temperature
- 12 Mount tissues on Superfrost Plus microscope slides



- 13 Allow slides to dry overnight
- 14 Coverslip with VectaShield Antifade Hard Set Mounting Medium (Cat#:H-1400)