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SPARC Adenosine 2A Receptor 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 adenosine 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:
Adenosine 2A Receptor: Mouse anti-A2A (Millipore #05-717)
Cholera toxin B-fragment: Goat anti-CT-B (Millipore #227040)
 - 1.1 Place 40um transverse spinal cord sections into 1xPBS-Triton (0.1%) in 12 well cell culture plates
 - 1.2 3x washes in 1xPBS-Triton (0.1%) for 5 minutes each at room temperature
 - 1.3 Antigen retrieval: place tissues into Heat-Induced Epitope Retrieval (TissuePro, Cat#: HIER01-32R) for 30 minutes at 85 degrees C
 - 1.4 3x washes in 1xPBS-Triton (0.1%) for 5 minutes each at room temperature
 - 1.5 Blocking: place tissues in 5% Normal Donkey Serum (NDS) in 1xPBS-Triton (0.1%) for 60 minutes at room temperature
 - 1.6 Primary Antibody Incubation: Incubate tissues in: 5%NDS in 1xPBS-Triton (0.1%), Mouse anti-A2A (1:1000), and Goat anti-Ct-B (1:2500) for 1 hour at room temperature
 - 1.7 Continue primary antibody incubation overnight at 4 degrees C
- 2 Day 2: secondary antibodies required:
AlexaFluor 488: donkey anti-goat (Invitrogen, Ref#A11055)
AlexaFluor 594: donkey anti-mouse (Invitrogen, Ref#A21203)
 - 2.1 3x washes in 1xPBS-Triton (0.1%) for 5 minutes each at room temperature
 - 2.2 Secondary Antibody Incubation: Incubate tissues in: 5%NDS in 1xPBS-Triton (0.1%), donkey anti-goat (1:1000), and donkey anti-mouse (1:500) for 2 hours at room temperature
 - 2.3 3x washes in 1xPBS for 5 minutes each at room temperature



- 3 Mount tissues on Superfrost Plus microscope slides
- 4 Allow slides to dry overnight
- 5 Coverslip with VectaShield Antifade Hard Set Mounting Medium (Cat#:H-1400)