Jul 12, 2019

SPARC Adenosine 2A Receptor Immunohistochemistry Protocol in Rat Tissues Labeled with Cholera Toxin B-fragment

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1 Works for me dx.doi.org/10.17504/protocols.io.2kfgctn

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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)