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Immunostaining Mouse Brain Tissue or Neuronal Cultures

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We use this protocol and it's working

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Abstract

This protocol describes immunohistochemical and immunoctyochemical methods to analyze protein expression in mouse brain. In the protocol, we describe the fixation of tissue or cultured neurons, the immunostaining procedure, and collecting images for analysis.

Protocol materials

Fluoromount-G **Southern Biotech** **Catalog #**0100-01

Troubleshooting

Tissue fixation

10m

1 Collection of fixed tissues slices from juvenile or adult mice

1.1 Anesthetize mouse, pin onto dissection tray and open chest cavity

1.2 Using a perfusion pump, pierce needle into left ventricle and sever the right atrium; immediately begin perfusing cold 1X PBS followed by 4% paraformaldehyde (PFA) in PBS

1.3 Dissect out brain into 15 mL tube containing cold 4% PFA in PBS

1.4 Incubate Overnight at 4 °C in 4% PFA in PBS

1.5 Incubate Overnight at 4 °C in 30% sucrose in PBS

1.6 Tissue can be kept in long-term storage at -80 °C ; when ready for IHC, slice 35 µm sections using a cryostat (Leica CM3050 S)

Equipment

Cryostat	NAME
Leica	BRAND
Cryostat	SKU
https://www.leicabiosystems.com/histology-equipment/cryostats/	LINK

1.7 Once slices are collected, IHC is performed on either free-floating sections (in cold 1X PBS) or slide-mounted sections

2 Fixation of cultured neurons

2.1 Aspirate culture media

2.2 Add solution containing 4% PFA and 4% sucrose in 1X PBS to culture dishes

2.3 Incubate for 00:10:00 at Room temperature 10m

2.4 Aspirate fixing solution and add 1X PBS

Tissue staining 10m

3 Wash samples three times for 10 minutes at Room temperature in 1X PBS on a rocker

4 Block and permeabilize for 2 hours at Room temperature in 1X PBS containing 4% normal goat serum (NGS) and 0.2% Triton X-100 on a rocker

5 Prepare primary antibody solution by diluting antibody at desired concentration (see step 5.1) in 1X PBS containing 2% NGS and 0.2% Triton X-100

5.1 Dilutions for antibodies used in Jain et al., 2023
- MAP2 (Chicken) - 1:2000
- TH (Mouse & Rabbit) - 1:1000
- All other primary antibodies - 1:500

6 Incubate in primary antibody overnight at 4 °C

7 Wash samples three times for 10 minutes at Room temperature in 1X PBS on a rocker

8 Prepare secondary antibody solution by diluting antibody at 1:1000 in 1X PBS containing 2% NGS and 0.2% Triton X-100

9 Incubate in secondary antibody for 1 hour at 4 °C in the dark

10 Wash samples three times for 10 minutes at Room temperature in 1X PBS on a rocker

11 If using free-floating sections, mount slices onto a slide.

For all sample types, mount coverslips using Fluoromount-G **Southern Biotech Catalog #**0100-01

12 Store samples at 4 °C for short-term storage or -20 °C for long-term storage

Imaging

13 Collect images of fixed tissue using a microscope:
- For high resolution (e.g. to trace axon sections): Nikon Ti Microscope, equipped with CSU-W1 spinning disk confocal, Plan Apo VC 100x/1.4 oil or 60x/1.4 oil objective and Andor Zyla sCMOS camera
- For whole brain sections: Nikon 6D conventional wide-field microscope, Plan Apo 20x/0.75 air objective and DS-Qi2 monochrome camera

14 Analyze images using ImageJ Software

14.1 In Jain et al. (2023), measurement of brain slices involved tracing one-pixel lines of dendrites and representative sections of axons. Fluorescence intensity of VMAT2-HA and TH per µm process length from 20 processes was used to determine the mean intensity per field.

14.2 In Jain et al. (2023), measurement of cultured neurons involved tracing one-pixel lines of dendrites and axons. Mean intensity per µm process length was determined in either three segments of the same axon or in three separate dendrites of the same neuron.