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Immunostaining Mouse Tissue

Forked from Immunostaining Mouse Brain Tissue or Neuronal Cultures

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Chelsie Steele[1], Yujie Fan[1]

[1]Caltech

Chelsie Steele
Caltech

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Protocol status: Working
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.

Materials

Buffers, Reagents, Materials

- 4% paraformaldehyde (diluted from Pierce™ 16% Formaldehyde (w/v), Methanol-free **Thermo Fisher Catalog #**28908) in 1X DPBS
- 1X DPBS
- 0.1 % Triton X-100 in DPBS
- Blocking Buffer: Normal Donkey Serum -Jackson Immune Research Labs - Cat No NC9624464
- DAPI - ThermoFisher Cat No D1306
- Prolonged Diamond Antifade Mountant - ThermoFisher Cat No P36965
- Appropriate primary and fluorophore-conjugated secondary antibodies
- Microscope slides

Troubleshooting

Collection of fixed tissues slices from mice

1d 0h 10m

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

1.1 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

1.2 Dissect out brain or wanted tissue into 15 mL tube containing cold 4% PFA in PBS

1.3 Incubate Overnight at 4 °C in 4% PFA in PBS 12h

1.4 Incubate Overnight at 4 °C in 30% sucrose in PBS 12h

1.5 Tissue can be kept in long-term storage at -80 °C ; when ready for IHC, slice 100 µm sections using a cryostat (Leica CM3050 S)

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

1.6 Once slices are collected, IHC is performed on free-floating sections in cold 1X DPBS with 5% Azide

Tissue staining

18h

2 Wash samples three times for 10 minutes at Room temperature in 1X DPBS + 0.1% Triton X-100 on a rocker 30m

3 Block and permeabilize for 2 hours at Room temperature in 1X DPBS containing 4% normal goat serum (NGS) and 0.1% Triton X-100 on a rocker 2h

4 Prepare primary antibody solution by diluting antibody at desired concentration in 1X DPBS containing 2% NGS and 0.1% Triton X-100

5 Incubate in primary antibody overnight at 4 °C 12h

6 Wash samples three times for 10 minutes at Room temperature in 1X DPBS+ 0.1% Triton X-100 on a rocker 30m

7 Prepare secondary antibody solution by diluting antibody at 1:1000 in 1X DPBS containing 2% NGS and 0.1% Triton X-100

8 Incubate in secondary antibody for 2 hours at 4 °C in the dark 2h

9 Wash samples three times for 10 minutes at Room temperature in 1X PBS + 0.1% Triton X-100 on a rocker 30m

10 Add DAPI 1:1000 to free floating samples for 10 minutes 10m

11 Wash samples two times for 10 minutes at RT in 1X DPBS + 0.1% Triton X-100 on a rocker 20m

12 Mount free floating slices onto a slide.

- For all sample types, mount using Prolonged Diamond Antifade Mountant

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

Imaging

14 Collect images of fixed tissue using a microscope:
- For high resolution: use Leica DMi8 Inverted Microscope with Spinning Disk