Nov 13, 2024

Immunohistology for Mouse Brain Sections

DOI
https://dx.doi.org/10.17504/protocols.io.5qpvo95yxv4o/v1

Richard Roth[1], Fuu-Jiun Hwang[1], Jun B. Ding[1], Yue Sun[1]

[1]Stanford University

Christine Weber-Schmidt
Allen Institute

DOI: https://dx.doi.org/10.17504/protocols.io.5qpvo95yxv4o/v1

Protocol Citation: Richard Roth, Fuu-Jiun Hwang, Jun B. Ding, Yue Sun 2024. Immunohistology for Mouse Brain Sections. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5qpvo95yxv4o/v1

Protocol status: Working
We use this protocol and it's working

Created: November 08, 2024

Last Modified: November 13, 2024

Protocol Integer ID: 111816

Keywords: ASAPCRN, immunohistology for mouse brain section, mouse brain tissue for immunostaining, preparing mouse brain tissue, mouse brain section, immunohistology, mouse brain, immunostaining, brain region, histology analysis, mouse, different antibody, antibody

Funders Acknowledgements:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-020551

Abstract

This protocol outlines the general steps for preparing mouse brain tissue for immunostaining and histology analysis. It can be adapted for different antibodies and brain regions as needed.

Attachments

DOCX

Ding_Lab_Immunohisto..

19KB

Materials

- Perfusion pump and tubing
- Surgical instruments
- Styrofoam plate
- PBS (Phosphate Buffered Saline)
- 4% Paraformaldehyde (PFA) in PBS
- 30% Sucrose in PBS
- Embedding medium: OCT (Optimal Cutting Temperature) compound
- Microtome
- Blocking solution: 1% w/v BSA, 10% Normal Donkey Serum (NDS), 0.1% Triton X-100 in PBS
- Primary antibody cocktail: Dilute primary antibodies at desired concentrations in blocking solution
- Secondary antibody cocktail: Dilute secondary antibodies at desired concentrations in PBS containing 1% BSA
- Mounting medium: Fluorescence-compatible mounting medium (e.g., Vector Laboratories #H-1500-10)
- Microscope slides and coverslips

Safety warnings

Wear appropriate PPE as required by your institution.

Ethics statement

Prior ethics approval (e.g. IACUC) should be obtained before performing these experiments. Approval was obtained by the Stanford University IACUC before any procedures were performed.

Transcardial Perfusion and Tissue Fixation

1 Prepare PBS and 4% PFA in PBS for perfusion.

2 Set up the perfusion pump and fill the tubing with PBS.

3 Anesthetize the mouse using isoflurane.

4 Pin the mouse to a Styrofoam plate.

5 Make a midline incision to expose the thoracic cavity, cutting through the skin and rib cage.

6 Insert the needle into the left ventricular chamber of the heart.

7 Switch on the perfusion pump and open the right atrium using sharp forceps immediately.

8 Perfuse the mouse with 10–15 mL of PBS to flush out the blood.

9 Switch the perfusion to 4% PFA and perfuse with 10–15 mL.

10 Dissect the brain and place it in 10 mL of 4% PFA for post-fixation for 1–3 hours at room temperature or overnight at 4°C.

11 Transfer the brain to a 30% sucrose solution in PBS at 4°C until the tissue sinks (24–48 hours). **Alternatively**, start with 15% sucrose in PBS until the tissue sinks (6–12 hours or overnight), then switch to 30% sucrose until it sinks (overnight or longer).

11.1 **Optional**: For long-term storage, embed the brain in OCT compound and freeze at -80°C.

Tissue Sectioning

12 Cool the microtome stage using dry ice.

13 Create a flat platform using OCT.

14 Attach the brain to the platform using a 1:3 mix of OCT and 30% sucrose in PBS.

15 Cut brain sections at 20–100 µm thickness and collect them in PBS.

15.1 **Optional**: For handling delicate 20 µm sections, you may mount them directly onto microscope slides and perform immunostaining on the slides.

15.2 **Optional**: If no antibody staining is required, proceed to Step 25 (mounting).

Immunostaining (Day 1)

16 Make PBS, permeabilization solution, blocking solution and primary antibody cocktail before or during incubation steps.

17 Wash sections 3 times for 10 minutes each in PBS.

18 Permeabilize the sections in 0.5% Triton X-100/PBS for 30 minutes at room temperature.

19 Wash sections twice for 5 minutes each in PBS.

20 Block sections in blocking solution for 1 hour at room temperature.

21 Add the primary antibody cocktail to the sections (placed in a 24-well plate or on a slide) and incubate overnight at 4°.

Immunostaining (Day 2)

22 Wash the sections 3 times for 10 minutes each in PBS.

23 Add the secondary antibody cocktail and incubate at room temperature for 2 hours, **protected from light**.

24 Wash the sections 3 times for 10 minutes each in PBS.

25 Mount sections using fluorescence-compatible mounting medium and carefully place coverslips.

26 Allow the slides to dry in the dark for 30 minutes to 1 hour at room temperature.

27 Store slides at 4°C, protected from light.

Microscopy

28 Analyze the stained sections using a fluorescence slide scanner or a confocal microscope.