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Immunohistochemistry (Histology + Imaging)-Mendelsohn et al 2025

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We use this protocol and it's working

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Abstract

This protocol outlines the steps for preparing mouse brain tissue for immunostaining and imaging from Mendelsohn et al 2025.

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 Columbia University IACUC before any procedures were performed.

Transcardial Perfusion and Tissue Fixation

1 Prepare PBS and ice cold 4% PFA (paraformaldehyde) in PBS for perfusion.

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

3 Deeply 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 mouse with PBS to flush out the blood.

9 Switch perfusion pump to the ice cold 4% PFA.

10 Dissect the brain and place it in 4% PFA for post-fixation overnight at 4 °C .

11 Wash brain x3 in PBS.

12 Transfer the brain to a 30% sucrose solution in PBS at 4 °C for 3 days.

13 Brains are embedded in OCT (Optimum Cutting Temperature Compound (Tissue-Tek)) then stored at -80 °C .

Tissue sectioning

14 Mount brain onto a cyrostat mount using OCT to be able to section in a coronal orientation.

15 Cut 50 µm sections and place sections in 24 well plates with PBS.

Staining (IHC)

2d 2h 15m

16 NOTE-stain sections in 24 well plates.
Dilute primary antibodies in PBST 0.3%, 0.5% BSA and 0.05% thimersol.

16.1 Primary Antibodies and concentrations are:
Rabbit anti-Foxp2 (Abcam, 1:10k)
Guinea Pig anti-Pou6f2 (Columbia, 1:2k)
Rabbit anti-GFP-488 (Invitrogen, 1:1k)
Chicken anti-GFP (Aves labs, 1:2k)
Rabbit anti-RFP (Rockland, 1:2k)
Rat anti-mCherry(Invitrogen, 1:2k)
Mouse anti-TH (Immunostar, 1:10k)
Chicken anti-Myc (Invitrogen, 1:250)

17 Incubate tissue sections with primary antibodies at 4 °C for 48:00:00 . 2d

18 Wash sections 3 times in PBS.

19 Incubate sections with secondary antibodies at a 1:1000 dilution for 02:00:00 at Room temperature . 2h
Use the below secondary antibodies pending primary antibodies used in sections.
Goat anti-Chicken 488 (Invitrogen)
Goat anti-Guinea Pig 568 (Invitrogen)
Goat anti-Mouse 488 (Invitrogen)
Goat anti-Mouse 568 (Invitrogen)

Goat anti-Rabbit 568 (Invitrogen)
Goat anti-Rabbit 647 (Invitrogen)
Goat anti-Rat 568 (Invitrogen)

20 Wash sections 3 times in PBS.

21 Stain for DAPI (1:1000 in PBS) at Room temperature for 00:15:00 . 15m

22 Mount sections onto Super- Frost Plus Slides with coverslips and desired mounting media.

Imaging

23 Pending experimental need visualize sections using:

A W1- Yokogawa inverted spinning disk confocal using a 10x or 20x objective.

OR

Automated high-throughput imaging of tissue sections was performed on a custom-built automated slide scanner using a AZ100 microscope equipped with a 4× 0.4NA Plan Apo objective
(Nikon Instruments Inc) and P200 slide loader (Prior Scientific), controlled by NIS-Elements using
custom acquisition scripts (Nikon Instruments Inc.).