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In Situ Hybridization -Mendelsohn et al 2025

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

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Abstract

This protocol outlines the steps for preparing mouse brain tissue for In Situ Hybridization from Mendelsohn et al 2025.

Attachments

ACD RNAscope Multipl...
1.1MB

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.

Before start

Read all steps and review materials needed before starting this protocol.

Transcardial Perfusion and Tissue Fixation

1 Prepare ice cold 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 Dissect the brain and place it in OCT (Optimum Cutting Temperature Compound (Tissue-Tek)) in an appropriate mold (avoid bubbles forming).

10 Rapidly freeze on dry ice then store at -80 °C .

Tissue sectioning

11 Mount brain onto a cyrostat mount to be able to section in a coronal orientation.

12 Cut 15µm sections and place sections on Super- Frost Plus Slides, then store at -80 °C .

In Situ Hybridization (ISH)

13 Fix sections to slides in 4% paraformaldyde (PFA) according to Advanced Cell Diagonstics (ACD) RNAscope Multiplex Fluorescent V2 Assay manual.

14 The next steps and visualization steps are detailed in the Advanced Cell Diagonstics (ACD) RNAscope Multiplex Fluorescent V2 Assay manual (in attachments of this protocol).