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Immunohistochemistry with Rodent Brain Sections

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We use this protocol and it's working

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Abstract

This protocol describes a detailed stepwise procedure of immunohistochemical analysis of rodent brain tissue, enabling detailed visualization of target antigens within regions of the hippocampus.

Guidelines

This protocol needs prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

Materials

Ketamine hydrochloride/xylazine hydrochloride solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**K113

Fixative: 4% Formaldehyde + 0.125% Glutaraldehyde in 0.1 M phosphate buffer

Formaldehyde solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**F8775-25ML +

Glutaraldehyde 50% EM Grade Aqueous **Electron Microscopy Sciences Catalog #**16320 in 0.1 M phosphate buffer (PB), warmed to 37 °C

Normal goat serum:

Normal Goat Serum **Jackson ImmunoResearch Laboratories, Inc. Catalog #**005-000-121

Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A4503

Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T8787

Primary antibodies: DDHD2 Polyclonal antibody **Proteintech Catalog #**25203-1-AP ,

Synapsin1/2 antibody **Synaptic Systems Catalog #**106 004

Alexa-conjugated secondary antibodies (Thermofisher Scientific)

ProLong™ Gold Antifade Mountant **Invitrogen - Thermo Fisher Catalog #**P36934

Nail polish (clear)

Equipments:

- Dissection tools
- Perfusion setup
- Vibratome

Procedure

4h 30m

1 Prepare the anesthetic cocktail (Ketamine/xylazine) and administer it to the mouse via intraperitoneal injection to achieve deep anesthesia.

> **Note**
>
> Confirm the absence of reflexes before proceeding.

2 Perform transcardial perfusion with 37 °C warmed fixative (4% formaldehyde + 0.125% glutaraldehyde in [M] 0.1 Mass Percent PB) until the liver and lungs blanch, indicating successful perfusion.

3 Carefully dissect the brain and immerse it in the same fixative Overnight at 4 °C.

4 Wash the fixed brain in [M] 0.1 Mass Percent PB to remove excess fixative.

5 Use a vibratome to cut coronal sections at 25 µm thickness. Collect sections in [M] 0.1 Mass Percent PB.

6 Prepare the blocking solution containing 2% normal goat serum, 2% BSA, 0.1 M PB, and 0.4% Triton X-100 and incubate brain sections in the blocking solution for 01:00:00 at Room temperature with gentle agitation.

1h

7 Dilute the primary antibodies in the blocking buffer and incubate the sections with the primary antibody solution Overnight at 4 °C.

1h

8 Wash the sections 3 times in 0.1 M PB, 10 minutes per wash, at room temperature.

8.1 Wash the sections in [M] 0.1 Mass Percent PB for 00:10:00 at Room temperature (1/3). 10m

8.2 Wash the sections in [M] 0.1 Mass Percent PB for 00:10:00 at Room temperature (2/3). 10m

8.3 Wash the sections in [M] 0.1 Mass Percent PB for 00:10:00 at Room temperature (3/3). 10m

9 Incubate the sections with Alexa-conjugated secondary antibodies prepared in the blocking buffer for 02:00:00 at Room temperature with gentle agitation in the dark to protect fluorescent dyes from photobleaching. 2h

10 Rinse the sections briefly in [M] 0.1 Mass Percent PB and mount on microscope slides.

11 Add ProLong Gold Antifade Reagent to each section and cover with a coverslip.

12 Seal the edges of the coverslip with nail polish to prevent drying.

13 Images were acquired with the Yokogawa spinning disk field scanning confocal system with microlensing (63x, oil immersion objective lens, CSU-W1 SoRa, Nikon) controlled by NIS elements (Nikon) software (RRID:SCR_014329).

Note

- Ensure all steps are performed with care to avoid tissue damage.
- Optimize primary and secondary antibody dilutions for specific targets.
- Protect fluorescently labeled sections from light exposure during and after staining.
- Perform controls to verify antibody specificity and avoid background staining.