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Mouse brain immunohistochemistry - colorimetric analysis

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

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Disclaimer

Abstract

This protocol details the mouse brain immunohistochemistry by colorimetric analysis.

Materials

- Euthatal
- PBS
- PFA 4% (v/v) in PBS
- Sucrose 30% (w/v) in PBS
- Quenching solution (3% H2O2, 10% Methanol in TBS)
- Blocking solution (5% normal goat serum, TBS-Triton 0.1%)
- Vector Elite ABC Kit **Catalog #**PK-4000
- 3,3′-diaminobenzidine (DAB, Sigma)
- Primary antibody

Before start

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

Procedure:

1d

1 Anesthetise the mice with Euthatal and perfuse with PBS and PFA 4%.

2 Fix the brains Overnight in 4% PFA at 4 °C, wash and left in 30% sucrose for 24:00:00 at 4 °C. 1d

3 Slice the brains into 35 µm-thick slices using a freezing microtome and store at -20 °C until processing for immunohistochemistry.

4 Rinse the free-floating sections (3X) with TBS for 10 min.

4.1 Rinse the free-floating sections with TBS for 00:10:00 (1/3). 10m

4.2 Rinse the free-floating sections with TBS for 00:10:00 (2/3). 10m

4.3 Rinse the free-floating sections with TBS for 00:10:00 (3/3). 10m

5 Incubate with quenching solution (3% H2O2, 10% Methanol in TBS) for 00:15:00. 15m

6 Subsequently rinse the sections (3X) in TBS for 10 min.

6.1 Subsequently rinse the sections in TBS for 00:10:00 (1/3). 10m

6.2 Subsequently rinse the sections in TBS for 00:10:00 (2/3). 10m

6.3 Subsequently rinse the sections in TBS for 00:10:00 (3/3). 10m

7 Incubate with blocking solution (5% normal goat serum, TBS-Triton 0.1%) for 01:00:00 at Room temperature . 1h

8 Perform the incubation with primary antibodies Overnight at 4 °C . 1h

9 The following day, rinse the sections (3X) with TBS Triton 0.1% for 10 min.

9.1 The following day, rinse the sections with TBS Triton 0.1% for 00:10:00 (1/3). 10m

9.2 The following day, rinse the sections with TBS Triton 0.1% for 00:10:00 (2/3). 10m

9.3 The following day, rinse the sections with TBS Triton 0.1% for 00:10:00 (3/3). 10m

10 Incubate with the secondary antibodies for 02:00:00 at Room temperature . 2h

11 Subsequently rinse the sections (3X) in TBS-Triton for 0.1% for 10 minutes.

11.1 Subsequently rinse the sections in TBS-Triton for 0.1% for 00:10:00 (1/3). 10m

11.2 Subsequently rinse the sections in TBS-Triton for 0.1% for 00:10:00 (2/3). 10m

11.3 Subsequently rinse the sections in TBS-Triton for 0.1% for 00:10:00 (3/3). 10m

12 Incubate for 02:00:00 at Room temperature with avidin-peroxidase complex (ABC kit, PK4000, Vector). 2h

13 Apply the 3,3′-diaminobenzidine (DAB, Sigma) to the slices to visualise Iba1 and DARPP-32 positive cells.

14 Obtain the images using a bright-field microscope (Macro/Micro Imaging System, Leica) under a 40X objective and analysed using Fiji.