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Mouse brain immunohistochemistry - colorimetric analysis

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Ilaria Morella¹, Riccardo Brambilla¹

¹Università di Pavia



Francesca Tonelli

MRC-PPU at The University of Dundee

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We use this protocol and it's working

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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 .
- 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).
- 4.2 Rinse the free-floating sections with TBS for  00:10:00 (2/3).
- 4.3 Rinse the free-floating sections with TBS for  00:10:00 (3/3).
- 5 Incubate with quenching solution (3% H₂O₂, 10% Methanol in TBS) for  00:15:00 .
- 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).
- 6.2 Subsequently rinse the sections in TBS for  00:10:00 (2/3).

1d



10m



10m



10m



15m



10m



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. 10m
- 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).
- 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.

2h

