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Immunohistochemistry

In 2 collections

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Peter Kilfeather[1]

[1]University of Oxford

Peter Kilfeather

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

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Abstract

This protocol describes the immunohistochemistry protocol.

Guidelines

This protocol needs prior approval by the users' Institutional Animal Care and Use Committee (IACUC)

Brain Preparation

1 Euthanize the mouse by lethal intraperitoneal injection of pentobarbital.

2 Upon loss of the pedal withdrawal reflex, dissect the thoracic cavity open.

3 Perfuse 25 mL phosphate-buffered saline (PBS) transcardially, followed by 25 mL 4 % paraformaldehyde (PFA), diluted in PBS.

Tissue Collection and Storage

4 Dissect and extract the brain.

5 Store the brain in 4 % PFA at 4 °C overnight.

Dehydration

6 Slice each brain into 3 mm coronal sections (approximately).

7 Pass each section through graded alcohol solutions (50, 70, 80, 95, 100, 100 and 100 % ethanol), holding for 10 minutes in each.

8 Transfer each section into xylene three times, for 20 minutes each.

9 Transfer each section into paraffin two times, for two hours each.

10 Embed each section in fresh paraffin, orienting as desired.

Sectioning

11 Take coronal sections at 7 µm using a Leica microtome (Leica Microsystems, Wetzlar, Germany) and mounted on glass slides (VWR, Superfrost Plus)

Deparaffinisation, Antigen Retrieval and Blocking

12 Transfer each slide-mounted set of sections through xylene (twice) and reversed graded alcohols (100, 95, 70, 50 %) for 10 minutes each.

13 Transfer to 1X citrate buffer (ab96678, Abcam) and perform antigen retrieval by microwave heat (20 minutes).

14 Block for one hour at room temperature using 10 % donkey serum in tris-buffered saline (TBS).

Primary Staining

15 Apply primary antibodies to TH (Abcam Cat# ab76442, RRID:AB_1524535, 1:500), GFP (Thermo Fisher Scientific Cat# A-11122, RRID:AB_221569, Invitrogen, 1:1000), CASR (Abcam Cat# ab79038, RRID:AB_2071489, 1:100) or TYROBP/DAP12 (Abcam Cat# ab283679, 1:100), prepared in 1 % donkey serum in TBS and incubate overnight at 4 °C

Secondary Staining

16 Wash slides in TBS three times, for five minutes each.

17 Incubate in secondary antibodies (Alexa Fluor-conjugated anti-rabbit/chicken A-31573, RRID:AB_2536183 and A78952, RRID:AB_2921074 1:1000) and DAPI (Thermo Fisher Scientific Cat# D3571, RRID:AB_2307445, 1:5000), prepared in TBS for 1 hour at room temperature.

18 Wash slides in TBS three times, for five minutes each.

19 Mount coverslips using FluorSave mounting medium (Millipore, Massachusetts, United States of America).