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IHC AD neuropathology protocol

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Immunohistochemical processing was conducted on tissue from chronically implanted animals, as well as non-implanted transgenic mice for characterization of AD neuropathology.

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Day 1 fluorescent IHC

1 Heat-induced antigen retrieval on all tissue at **60 °C** for 2 hours in phosphate buffer (PB).

2 Wash sections 3 x 10min in PB containing 0.2 % Triton X-100 (PBT+).

3 Block sections using 5 % normal goat serum in PBT+ for 1 hour.

4 Incubate sections with the primary antibodies Iba1 (1:1000; ab15690, Abcam, Cambridge, UK), McSA1 (1:1000; MediMabs, Montreal, Canada; characterization), and Aβ42 (1:1000; IBL America, Minnesota, USA; characterization) in PBT+ for 4 hours at **4 °C** .

Day 1 DAB

5 Heat-induced antigen retrieval on all tissue at **60 °C** for 2 hours in phosphate buffer (PB).

6 Wash in PB for 2x 10 minutes.

7 Permeabilize with 0.5 % Triton-X-100 in Tris-buffered saline (TBS-Tx; 50 mm Tris, 150 mm NaCl, pH 8.0) for 10 minutes.

8 Block with 10 % normal goat serum in TBS-Tx for 30 minutes.

9 Incubate with the primary antibody (AT8, 1:1000) in TBS-Tx overnight at **4 °C**

Day 2 fluorescent IHC

10 Wash sections for 3 x 10 minutes with PBT.

11 Incubate sections with Alexa Fluor 546 to visualize Iba1 and McSA1, and Alexa Fluor 488 to visualize Aβ42, for 2 hours at room temperature, protected from light.

12 Wash sections for 10 minutes with 4 ', 6-diamidino-2-fenylindol (DAPI; 1:10 000; Sigma-Aldrich, Saint-Louis, MO, USA) and PB, followed by 3 washes for 10 minutes with PB.

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Day 2 DAB

13 Wash sections with TBS-Tx for 3 × 10 minutes.

14 Incubate with a biotinylated goat anti-mouse secondary antibody (1:500; Sigma-Aldrich, St Louis, MO, USA) in TBS-Tx for 90 minutes.

15 Wash sections for 3 x 10 minutes with TBS-Tx.

16 Incubate with ABC (Vectastain ABC kit, Vector Laboratories, Burlingame, CA, USA) for 90 minutes.

17 Wash with TBS-Tx for 3 x 10 minutes.

18 Wash with Tris-HCl for 2 × 5 minutes.

19 Incubate tissue with 0.67 % diaminobenzidine (DAB) and 0.024 % H2O2 for 10 minutes before a final wash with Tris-HCl (2 × 5 minutes).

Mounting sections

20 Mount tissue on cut edge frosted glass slides (VWR International, Radnor, PA, USA) with Tris-HCl and leave to dry for at least 4 hours on a **38 °C** heating plate, protected from light.

21 Place mounted tissue in Xylene (VWR International, Radnor, PA, USA) for at least 5 minutes for defatting and to remove excess water from the tissue, and then coverslip with Entellan (VWR International, Radnor, PA, USA) containing Xylene.

22 Leave the mounted tissue to dry overnight, protected from light.

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