

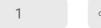


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IHC AD neuropathology protocol

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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).
- 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.

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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 δ 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.
- 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 8 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.

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 % H202 for 10 minutes before a final wash with Tris-HCl (2×5 minutes).

Mounting sections

- 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.