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Immunoperoxidase detection of human alpha-synuclein in mouse brain



Forked from [ChAT and human alpha-synuclein immunofluorescence staining](#)

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Protocol status: Working

We use this protocol and it's working



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Abstract

This protocol is designed for detecting human alpha-synuclein (Abcam, RRID:AB_2537217, MJFR1). Tissues stained with this protocol include 35 µm free-floating mouse brain sections. All tissues were from mice perfused with 4% PFA.

Immunoperoxidase detection of human alpha-synuclein in mouse brain samples

- 1 Wash tissue slices in tris-buffer saline (TBS: 0.05M Trizma base and 0.15M NaCl; pH: 7.6) for 10 minutes. Repeat x3.
- 2 Incubate in in 10% Methanol + 3% H₂O₂ in TBS for 20 min at room temperature
- 3 Wash tissue slices in tris-buffer saline (TBS: 0.05M Trizma base and 0.15M NaCl; pH: 7.6) for 10 minutes. Repeat x3.
- 4 Incubate in blocking buffer (5% goat serum, 2% BSA, 0.5% triton X-100 in TBS) for 1 hour at room temperature.
- 5 Incubate with anti-human-alpha-synuclein primary antibody (RRID:AB_2537217) at 1:5000 dilution in 50% blocking solution for 24 hours at room temperature.
- 6 Wash tissue slices in TBS-T (TBS+ 0.25% Triton x-100) for 10 minutes. Repeat x3.
- 7 Incubate in goat anti-rabbit biotinylated secondary antibody at 1:250 dilution in 50% blocking buffer for 1 hour at room temperature.
- 8 Wash tissue slices in TBS-T(TBS+ 0.25% Triton X-100) for 10 minutes. Repeat x 2.
- 9 Wash tissue slices in TBS (0.05M Trizma base and 0.15M NaCl; pH: 7.6) for 10 minutes.
- 10 Incubate 1 hour with ABC complex. ABC complex to be prepared 30 min prior to use.
- 11 Wash tissue slices in TBS-T(TBS+ 0.25% Triton X-100) for 10 minutes. Repeat x 2.
- 12 Wash tissue slices in TBS (0.05M Trizma base and 0.15M NaCl; pH: 7.6) for 10 minutes.
- 13 Stain with the peroxidase chormogen diaminobenzidine



- 14 Wash tissue slices in TBS (0.05M Trizma base and 0.15M NaCl; pH: 7.6) for 10 minutes. Repeat X 3.