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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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Pietro La Vitola[1]

[1]German Center for Neurodegenerative Diseases (DZNE)

P Pietro La Vitola
German Center for Neurodegenerative Diseases (DZNE), Alignin...

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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.