

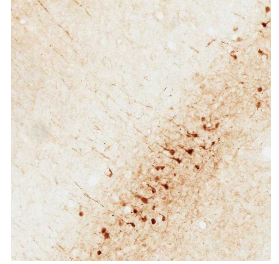


Feb 22, 2024

🌐 Protocol for "immunoperoxidase detection of alpha-synuclein in non-human primate brain samples"

DOI

<https://dx.doi.org/10.17504/protocols.io.8epv5x4wdg1b/v1>



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DOI: <https://dx.doi.org/10.17504/protocols.io.8epv5x4wdg1b/v1>

Protocol Citation: jlanciego 2024. Protocol for "immunoperoxidase detection of alpha-synuclein in non-human primate brain samples. [protocols.io https://dx.doi.org/10.17504/protocols.io.8epv5x4wdg1b/v1](https://dx.doi.org/10.17504/protocols.io.8epv5x4wdg1b/v1)

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Protocol status: Working

We use this protocol and it's working

Created: February 22, 2024

Last Modified: February 22, 2024

Protocol Integer ID: 95605

Keywords: Immunoperoxidase, diaminobenzidine, ABC method, immunohistochemistry, brain, synuclein in the macaque brain, immunoperoxidase detection of neuron, synuclein at the level, synuclein, immunoperoxidase detection, immunoreactivity for alpha, macaque brain, showing immunoreactivity, neuron, cerebral cortex

Funders Acknowledgements:

Aligning Science Across Parkinson's through the Michael J. Fox Foundation for Parkinson's Research

Grant ID: ASAP-020505

Disclaimer

Nothing to be disclosed

Abstract

Here an step-by-step protocol is provided summarizing the immunoperoxidase detection of neurons expressing alpha-synuclein in the macaque brain. Enclosed image illustrates layer V pyramidal neurons showing immunoreactivity for alpha-synuclein at the level of the cerebral cortex (insular gyrus).

Immunoperoxidase protocol (all steps below are conducted in free-floating sections and at room temperature unless otherwise noted)

- 1 Frozen coronal sections (40 microns-thick) to be obtained in a sliding microtome (Leica HM400) and collected in 0.125 M phosphate buffer (PB) pH 7.4 as 10 series of adjacent sections.
- 2 Wash the sections three times (10 min each) in phosphate-buffered saline solution (PBS; 0.05M pH 7.4)
- 3 Inactivate the endogenous peroxidase activity: incubation for 40 min in a solution made of 10 ml ethanol, 600 microliters of H₂O and 133 microliters of H₂O₂.
- 4 Preincubation solution (60 min): 1% bovine serum albumin (BSA) + 0.05% triton X-100 + 0.05% gelatin.
- 5 Incubation with the primary antibody (overnight at 4 °C). Mouse anti-alpha-synuclein (monoclonal), 1:40 in PBS.
- 6 Wash the sections three times (10 min each) in PBS 0.05M pH 7.4
- 7 Incubation with the secondary antibody (120 min). Donkey anti.mouse (Biotin-SP), 1:600 in PBS.
- 8 Wash the sections three times (10 min each) in PBS 0.05M pH 7.4
- 9 Incubation with ABC complex (90 min). ABC complex to be prepared 30 min prior to use.
- 10 Wash the sections three times (10 min each) in PBS 0.05M pH 7.4
- 11 Stain with the peroxidase chromogen diaminobenzidine (DAB; 3-5 min). Incubation solution made of 0.05% DAB and 0.01% H₂O₂ in PBS 0.05M pH 7.4
- 12 Wash the sections three times (10 min each) in PBS 0.05M pH 7.4



- 13 Mount free-floating sections in gelatin-coated slides and air-dry overnight.
- 14 Sections to be dehydrated and cleared in 100% toluene (2 × 10 min) and coverslipped with DePex.