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

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## 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).

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**Protocol status:** Working  
We use this protocol and it's working

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## Immunoperoxidase protocol (all steps below are conducted in free-floatin...

- 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 H<sub>2</sub>O and 133 microliters of H<sub>2</sub>O<sub>2</sub>.
- 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 Wahs 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 chormogen diaminobenzidine (DAB; 3-5 min). Incubation solution made of 0.05% DAB and 0.01% H<sub>2</sub>O<sub>2</sub> in PBS 0.05M pH 7.4
- 12 Wash the sections three times 810 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 x 10 min) and coverslipped with DePex.