

NBP1-19796 Protocol

Protocol specific for Monoamine Oxidase A Antibody (NBP1-19796)

Protocol specific for Monoamine Oxidase A Antibody (NBP1-19796) Immunohistochemistry (formalin fixed paraffin-embedded sections)

I. Deparaffinization/Rehydration

- 1.1. Heat slides in an oven at 65 degrees C for 1 hour.
- 1.2. De-paraffinize/hydrate using the following series of washes: two Xylene washes (3 min each), followed by two 100% ethanol rinses (3 min each), followed by 95% ethanol, 70% ethanol, 50% ethanol, 30% ethanol, followed by TBST wash for 3 min on a shaker.

II. Antigen Retrieval

This is recommended Heat Induced Epitope Retrieval (HIER) using Decloaking Chamber/Pressure Cooker. Hot water bath or Microwave with temperature sensor can be also used (protocol would vary depending on the method used).

- 2.1. Add 500 ml of dH 2O to Decloaker/Pressure Cooker.
- 2.2. Immerse slides into staining dish containing Antigen Retrieval Solution. Place staining dish into decloaking chamber.
- 2.3. Program to run for 30 seconds at 125 degrees C, followed by 10 seconds at 90 degrees C.
- 2.4. Let it cool down to room temperature (10 - 20 minutes).
- 2.5. Removes slides and rinse in TBST.
- 2.6. Proceed to Staining step.

III. Staining

- 3.1. Wash slides with TBST for 3 min on a shaker.
- 3.2. Inactivate endogenous peroxidase by covering tissue with 3% hydrogen peroxide for 5 min.
- 3.3. Wash slides three times with TBST (3 min each on a shaker).
- 3.4. Block slides with the blocking solution for 1 hour.
- 3.5. Dilute NBP1-19796 1:50-1:200.
- 3.6. Apply NBP1-19796 to each section and incubate overnight in the humidified chamber (4 degrees C).
- 3.7. Wash slides three times with TBST (3 min each on a shaker).
- 3.8. Apply to each section secondary HRP-conjugated anti-rabbit antibody diluted in the blocking solution per manufacturer's recommendation; incubate for 30 min at room temperature.
- 3.9. Wash slides three times with TBST (5 min each on a shaker).
- 3.10. Add freshly prepared DAB substrate to the sections and incubate until stain develops (generally 1 min).
- 3.11. Rinse sections with water.
- 3.12. Counterstain with Hematoxylin (generally 10 seconds).
- 3.13. Rinse sections with water.
- 3.14. Dehydrate samples using two washes with 100% Ethanol (3 min each), followed by two rinses with Xylene (3 min each).
- 3.15. Mount coverslips on slides using permanent mounting medium.