

May 03, 2020

# SPARC\_Duke\_Grill\_OT2-OD025340\_VagusNerve\_IHC\_TH

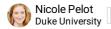
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#### ABSTRACT

The protocol describes immunohistochemistry with anti-tyrosine hydroxylase, as it has been applied to cervical and abdominal vagus nerve samples from rats, pigs, and humans.

#### MATERIALS TEXT

- Microscope slides with paraffin slices
- Xylene
- Ethanol
- Deionized water
- HIER Buffer L (Thermo, TA-135-HBL)
- H2O2
- Tris buffer
- Tris Tween buffer
- DAKO Protein Block (X0909)
- Antibody Diluent OP Quanto (Thermo, TA-125-ADQ)
- Rabbit anti-tyrosine hydroxylase (Abcam, ab112)
- Biotinylated SP-conjugated Affinipure goat anti-rabbit IgG (H+L) (Jackson, 111-065-144)
- ABC Elite (Vector, PK-6100)
- DAB chromogen (Thermo, TA-125-QHDX)
- Harris hematoxylin (Thermo, 6765003)
- DPX mountant (Electron Microscopy Sciences, 13512)
- Microscope with color camera

## Immunohistochemistry

- 1 Bake slides with sections of paraffin-embedded vagus nerve overnight at 50oC and then cool overnight.
- Deparaffinize the slides and hydrate them to distilled water: xylene (2x 6 min), 100% ethanol (5 min), 95% ethanol (4 min), 70% ethanol (3 min), deionized water (2x 1 min).
- 3 Perform heat-induced epitope retrieval (HIER) at 120oC for 30 s followed by 90oC for 10 s, using a buffer with pH 6.0 (Thermo, TA-135-HBL).
- 4 Cool for 20 min at room temperature.
- 5 Rinse in deionized water (2x 2 min).
- 6 Block with 3% H2O2 diluted in deionized water for 10 min.

| 7  | Rinse in deionized water (2x 2 min).   |
|----|--|
| 8  | Rinse in Tris buffer (1x 2 min).   |
| 9  | Block using DAKO Protein Block (X0909) for 10 min at room temperature.   |
| 10 | Apply the primary antibody (rabbit anti-tyrosine hydroxylase, Abcam, ab112) diluted in Thermo Antibody Diluent to a concentration of 1:250, and incubate overnight at 4oC.   |
| 11 | Rinse in Tris Tween buffer (2x 2 min).   |
| 12 | Rinse in Tris buffer (1x 2 min).   |
| 13 | Apply the secondary antibody (biotinylated SP-conjugated Affinipure goat anti-rabbit IgG (H+L), Jackson, 111-065-144) diluted in Thermo Antibody Diluent to a concentration of 1:500, and incubate for 1 hour at room temperature. |
| 14 | Rinse in Tris Tween buffer (2x 2 min).   |
| 15 | Rinse in Tris buffer (1x 2 min).   |
| 16 | Apply ABC Elite (Vector, PK-6100) at a concentration of 1:50 for 30 min at room temperature.   |
| 17 | Rinse in Tris Tween buffer (2x 2 min).   |
| 18 | Rinse in Tris buffer (1x 2 min).   |
| 19 | Apply DAB chromogen (Thermo, TA-125-QHDX) for 3 min at room temperature.   |

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| 20         | Rinse in | deionized | warer | ( / X / | TTHEFT. |
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- 21 Counterstain using hematoxylin.
- $22 \quad \text{ Dehydrate, clear, and coverslip using DPX mountant.} \\$

### Microscopy

Each sample was imaged at 20x using a Nikon Ti2 microscope with a Photometrics Prime 95B-25MM camera (Nikon Instruments Inc.). We selected the best of four slices for each sample based on the quality of the slice (no tearing or fraving).