



SPARC_Duke_Grill_OT2-OD025340_VagusNerve_IHC_TH V.2

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Version 2

Jul 23, 2021

1 Works for me



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dx.doi.org/10.17504/protocols.io.bwuwpexe

SPARC

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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.

DOI

dx.doi.org/10.17504/protocols.io.bwuwpexe

PROTOCOL CITATION

J. Ashley Ezzell, Nicole A. Pelot, Kara A. Clissold, Warren M. Grill 2021. SPARC_Duke_Grill_OT2-OD025340_VagusNerve_IHC_TH. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bwuwpexe>
Version created by Nicole Pelot

KEYWORDS

Vagus nerve, peripheral nerve, immunohistochemistry, tyrosine hydroxylase, TH, sympathetic hitchhikers

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CREATED

Jul 23, 2021

LAST MODIFIED

Jul 23, 2021

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

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Immunohistochemistry

- 1 Bake slides with sections of paraffin-embedded vagus nerve overnight at 50oC and then cool overnight.
- 2 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 4°C.
- 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.

20 Rinse in deionized water (2x 2 min).

21 Counterstain using hematoxylin.

22 Dehydrate, clear, and coverslip using DPX mountant.

Microscopy

23 Each sample was imaged at 20x using a Nikon Ti2 microscope with a DS-Ri2 color CMOS camera (Nikon Instruments Inc.). We selected the best of four slices for each sample based on the quality of the slice (no tearing or fraying).