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SPARC_Duke_Grill_OT2-OD025340_VagusNerve_IHC_ChAT

Forked from SPARC_Duke_Grill_OT2-OD025340_VagusNerve_IHC_ChAT

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1 Works for me dx.doi.org/10.17504/protocols.io.buw6nxhe

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ABSTRACT

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

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PROTOCOL CITATION

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FORK NOTE

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KEYWORDS

Vagus nerve, peripheral nerve, immunohistochemistry, choline acetyltransferase, ChAT, vagal efferents

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MATERIALS TEXT

- Microscope slides with paraffin slices
- Xylene
- Ethanol
- Deionized water
- HIER Buffer L (Thermo, TA-135-HBL) for human samples or BioCare Borg Decloaker (BD1000MM) for rat/pig samples
- H2O2
- Tris buffer
- Tris Tween buffer
- DAKO Protein Block (X0909)
- Antibody Diluent OP Quanto (Thermo, TA-125-ADQ)
- Goat anti-choline acetyltransferase (Millipore, AB144P)
- Biotinylated SP-conjugated Affinipure donkey anti-goat IgG (H+L) (Jackson, 705-065-147)
- 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

DISCLAIMER:

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 (human) or at 95oC for 5 min (rat, pig) followed by 90oC for 10 s, using a buffer with pH 6.0 (Thermo, TA-135-HBL) for human samples or a buffer with pH 9.0 (BioCare, BD1000MM) for rat/pig samples.

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.

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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 (goat anti-choline acetyltransferase, Millipore, AB144P) diluted in Thermo Antibody Diluent to a concentration of 1:100 for cervical & abdominal human samples, 1:50 for cervical pig samples, 1:100 for abdominal pig samples, and 1:200 for cervical & abdominal rat samples. 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 donkey anti-goat IgG (H+L), Jackson, 705-065-147) 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) at room temperature for 2.5 min for cervical & abdominal human samples, 2 min for cervical pig samples, 3 min for abdominal pig samples, and 4 min for cervical & abdominal rat samples.

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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).

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