



May 11, 2021

SPARC_Duke_Grill_OT2- OD025340_VagusNerve_IHC_ChAT

Forked from [SPARC_Duke_Grill_OT2-OD025340_VagusNerve_IHC_ChAT](#)J. Ashley Ezzell¹, Nicole A. Pelot¹, Kara A. Clissold¹, Warren M. Grill¹¹Duke University**1** Works for me dx.doi.org/10.17504/protocols.io.buw6nxheNicole Pelot
Duke University

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.

DOI

dx.doi.org/10.17504/protocols.io.buw6nxhe

PROTOCOL CITATION

J. Ashley Ezzell, Nicole A. Pelot, Kara A. Clissold, Warren M. Grill 2021. SPARC_Duke_Grill_OT2-OD025340_VagusNerve_IHC_ChAT. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.buw6nxhe>



FORK NOTE

FORK FROM

[Forked from SPARC_Duke_Grill_OT2-OD025340_VagusNerve_IHC_ChAT, Nicole Pelot](#)

KEYWORDS

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

LICENSE

————— This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

May 11, 2021

LAST MODIFIED

May 11, 2021

PROTOCOL INTEGER ID

49854

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:

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

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.

- 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 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 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.

- 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).