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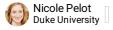
© SPARC_Duke_Grill_OT2-OD025340_HumanVagusNerve_Claudin1IHC_Morphology V.2

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

The protocol describes immunohistochemistry with anti-claudin-1, imaging, image segmentation, and image analysis methods to quantify human vagus nerve morphology.

PROTOCOL CITATION

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KEYWORDS

Vagus nerve, nerve morphology, human vagus nerve, claudin-1, endoneurium, perineurium, epineurium, fascicles, image segmentation

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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-claudin-1 (Abcam, ab15098)
- 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
- Nikon's NIS Elements
- Matlab

DISCLAIMER:

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mmunohistochemistry		
1	Bake slides with sections of paraffin-embedded vagus nerve at 50oC 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-claudin-1, Abcam, ab15098) diluted in Thermo Antibody Diluent to a concentration of 1:50, 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 1 min at room temperature.
20	Rinse in deionized water (2x 2 min).
21	Counterstain using hematoxylin.
22	Dehydrate, clear, and coverslip using DPX mountant.
Microsc	ору

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Each sample was imaged at 10x using a Nikon Ti2 microscope with a Photometrics Prime 95B-25MM camera (Nikon

23 Each

Instruments Inc.). We selected the best of four slices for each sample based on the quality of the slice (no tearing or fraying).

Image Segmentation

- We used Nikon's NIS Elements software (v5.02.01, Build 1270) to segment human vagus nerve immunohistochemical micrographs (anti-claudin-1) using the General Analysis RGB tool.
- 25 For each image, we selected preprocessing steps, such as smoothing and sharpening.
- For each image, we selected ranges of hues, saturations, and intensities to values that identify the perineurium and different values to identify the entirety of the nerve.
- For each image, we selected postprocessing steps, such as setting a minimum size criterion (eliminate small off-target regions), smoothing, cleaning, closing, and filling holes.
- We made manual adjustments as needed, including manual deletion of off-target regions and filling of target areas that had not been captured.
- 29 We converted the binary segmented image into "Graticule Masks", binary images saved as TIFs.

Image Analysis

- 30 We imported the TIFs into Matlab and generated a data structure of the x and y coordinates of the pixels for each closed boundary of the loaded binary images using the *bwboundaries* function.
- 31 We stored the pixel coordinates with indexing that assigns a fascicle number, which were then checked so that both the interior and exterior perineurium trace relate to the same fascicle.
- 32 We scaled the pixel coordinates to microns using the segmented scale bar.
- We calculated cross-sectional area of each fascicle (inner perineurium and outer perineurium traces) and nerve using Matlab's polyarea. Effective diameter (for a nerve or fascicle) is the diameter of the circle that has the same cross-sectional area as the raw trace. The perineurium thickness is half of the difference in effective diameters of the inner and outer perineurium traces.