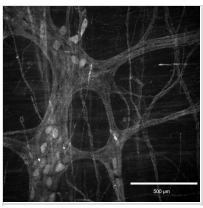

Feb 08, 2022

Immunohistochemistry of porcine enteric neurons

Gemma Mazzuoli-Weber[1], Michael Schemann[2], Kristin Elfers[1], Birgit Kuch[3], Susanne Hoppe[4]

[1]University of Veterinary Medicine Hannover, Foundation, Germany;

[2]Techical Univeristy of Munich, Germany; [3]Technical University of Munich, Germany;

[4]Institute for Physiology and Cell Biology University of Veterinary Medicine Hannover, Foundation

1

dx.doi.org/10.17504/protocols.io.b4qrqvv6

SPARC
Tech. support email: **info@neuinfo.org**

Manmeet Bains

This protocol is to investigate the presence and localization of neurochemical markers in the submucosal and myenteric plexus of the porcine colon using immunhistochemical staining techniques.

DOI

dx.doi.org/10.17504/protocols.io.b4qrqvv6

Gemma Mazzuoli-Weber, Michael Schemann, Kristin Elfers, Birgit Kuch, Susanne Hoppe 2022. Immunohistochemistry of porcine enteric neurons. **protocols.io** https://dx.doi.org/10.17504/protocols.io.b4qrqvv6

protocol ,

Feb 04, 2022

Feb 08, 2022

57841

Citation: Gemma Mazzuoli-Weber, Michael Schemann, Kristin Elfers, Birgit Kuch, Susanne Hoppe Immunohistochemistry of porcine enteric neurons https://dx.doi.org/10.17504/protocols.io.b4qrqvv6

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium,

- samples of pocine colon
- Krebs solution for preparation containing in (mM): 117 NaCl, 11 Glucose, 4.7 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$; Carl Roth GmbH & Co. KG (Karlsruhe, Germany)
- Sylgard® 184; World Precision Instruments (Sarasota, FL, USA)
- 4% paraformaldehyde; Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany)
- 0.002% picric acid; Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany)
- 0.1 mol/l phosphate buffer; Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany)
- phosphate-buffered saline (PBS): H_2O, Sodium Phosphate Monobasic, Sodium Phosphate Dibasic, NaCl; Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany)
- 0.1% NaN_3; Carl Roth GmbH & Co. KG (Karlsruhe, Germany)
- 4% horse serum; Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany)
- Triton X-100; Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany)
- object slides; Gerhard Menzel B. V. & Co. KG (Braunschweig, Germany)
- glycerol; Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany)
- cover slips; Omnilab-Laborzentrum GmbH & Co. KG (Gehrden, Germany)
- all antibodies from table 1 and table 2
- epifluorescence microscope; Olympus Corporation (Hamburg, Germany)
- Olympus cellSens Standard Software, Olympus Corporation (Hamburg, Germany)

1 Samples of colon were taken from apparently healthy pigs, placed in ice-cold oxygenated Krebs solution for preparation and immediately transferred to the laboratory. Tissues were then dissected in the ice-cold oxygenated Krebs solution for preparation to obtain whole-mount inner submucosal plexus or myenteric plexus preparations.

2 Tissue specimens were fixed overnight in a solution containing 4% paraformaldehyde and 0.002% picric acid in 0.1 mol/l phosphate buffer and then washed (3 x 10 min) in PBS.

3 The preparations were then incubated in PBS/NaN33/horse serum for 1h at room temperature followed by 12h and 2h incubation with the primary and secondary antibodies, respectively.

4 Specimens were washed in PBS, mounted on object slides and cover slipped with a solution of PBS (pH 7.0)/NaN3 containing 65% glycerol.

5 The preparations were examined with an epifluorescence microscope equipped with appropriate filter blocks. Pictures were acquired with a camera connected to a computer and controlled by Olympus cellSens Standard Software.

6 Pictures of the stained ganglia were acquired.

Citation: Gemma Mazzuoli-Weber, Michael Schemann, Kristin Elfers, Birgit Kuch, Susanne Hoppe Immunohistochemistry of porcine enteric neurons https://dx.doi.org/10.17504/protocols.io.b4qrqvv6

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium,