APR 28, 2021

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.w3tfgnn

Protocol Citation: Josiane Fakhry, Martin Stebbing, Billie Hunne, John B. Furness 2021. Immunohistochemistry and high resolution microscopy of human gastric enteroendocrine cells.
protocols.io
<https://dx.doi.org/10.17504/protocols.io.w3tfgnn>

MANUSCRIPT CITATION:
<https://doi.org.ezp.lib.unimelb.edu.au/10.1007/s00441-018-2957-0>

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

Protocol status: Working
 We use this protocol and it's working

Created: Jan 14, 2019

Last Modified: Apr 28, 2021

Immunohistochemistry and high resolution microscopy of human gastric enteroendocrine cells

Josiane Fakhry¹, Martin Stebbing², Billie Hunne¹, John B. Furness²

¹University of Melbourne;

²University of Melbourne and Florey Institute of Neuroscience and Mental Health

SPARC

Tech. support email: info@neuinfo.org

Martin Stebbing

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and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to [protocols.io](#) is not peer reviewed and may not have undergone a formal approval of any kind.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.

ABSTRACT

Enteroendocrine cells are important regulators of gastrointestinal, digestive and metabolic function. Here we describe protocols for identifying, mapping and characterising these cells in the human gastric mucosa using immunohistochemistry and superresolution microscopy techniques.

Keywords: Oxyntic gland,
Gastrointestinal hormones,
Ghrelin, 5-Hydroxytryptamine,
Somatostatin, Pancreastatin

MATERIALS

⊗	Mouse Anti-Calbindin Antibody RRID:AB_2314069 Swant Catalog #18F
⊗	Rabbit anti-5-HT antibody AB_572263 Immunostar Catalog #20080
⊗	Goat anti-5-HT antibody AB_572262 Immunostar Catalog #20079
⊗	Mouse anti Gastrin/CCK RRID: AB_2650429 Contributed by users Catalog #28.2
⊗	Chicken anti-Ghrelin antibody RRID: AB_2041392 Abcam Catalog #Ab15861
⊗	Rabbit anti-GLP1 antibody RRID: AB_2650430 Contributed by users Catalog #8912
⊗	Mouse anti-mast cell tryptase antibody - RRID:AB_2206479 Millipore Catalog #MAB 1222
⊗	Rabbit anti-Gastrin antibody - RRID: AB_2762851 Contributed by users Catalog #8007
⊗	Chicken anti-PYY antibody RRID: AB_1855196 Sigma Aldrich Catalog #GW22771
⊗	Rabbit anti-Calbindin antibody Contributed by users Catalog #R202
⊗	Rabbit anti-Ghrelin antibody RRID: AB_2767291 Contributed by users Catalog #RY1601

Addition antibodies (no supplier):

Rabbit anti-Gastrin 8007 (gift from Dr Jens Rehfeld) RRID:AB_2762851

- 1 Stomach regions were collected from six patients who were undergoing gastric sleeve surgery for obesity at the Renown Regional Medical Center, Reno, Nevada. Resections were of the full greater curvature (from the fundus to the antrum) from male and female patients between the ages of 48 and 60 who were non-diabetic. The tissue was placed in cold fixative (2% formaldehyde plus 0.2% picric acid in 0.1 M sodium phosphate buffer, pH 7.0) kept overnight at 4 °C. Tissues were then washed three times (10 min) with dimethyl sulfoxide (DMSO) and then three times (10 min) with PBS. Tissue samples were then transferred to PBS-azide and sent to the University of Melbourne. The tissue samples were placed in 50% PBS-sucrose-azide and 50% OCT mixture (Tissue Tek, Elkhart, IN, USA) for 24 h, before being trimmed, embedded in 100% OCT and frozen in isopentane cooled with liquid nitrogen.
- 2 Sections of 12 µm thickness were cut, allowed to dry at room temperature for 1 h on microscope slides (SuperFrost Plus®; Gracle Scientific, Victoria, Australia) and incubated with 10% normal horse serum plus 1% Triton X-100 in PBS for 30 min. Mixtures of primary antibodies (Table 1) for double staining were then placed on the sections that were left at 4 °C overnight. The tissue was washed three times in PBS and incubated with appropriate secondary antibodies labelled with Alexa Fluor dyes for 1 h at room temperature. To help reduce background fluorescence, tissue was washed with PBS for 5 min and then incubated with a quenching buffer (5 mM CuSO₄, 50 mM ammonium acetate, pH 5) for 30 min at room temperature. Preparations were then

washed three times with PBS for 10 min. Preparations were washed twice with distilled water for 5 min then incubated for 5 min with bisbenzimide (blue), diluted 10 µg/mL in dH₂O, to stain nuclei. Sections were then washed three times with distilled water before mounting with non-fluorescent mounting medium (Dako, Carpinteria, CA, USA). An absorption test was applied to test the specificity of ghrelin antibodies binding to cells in the human stomach. The diluted anti-ghrelin antibodies were equilibrated with human ghrelin peptide (100 nM to 100 µM) for 24 h at 4 °C before being used for staining of tissue sections as above. There was a concentration-related reduction in the immunohistochemical localisation. The immunoreactivity using chicken anti-ghrelin 14481 was reduced with 100 nM and 1 µM peptide and was abolished with 10 µM and 100 µM. Immunoreactivity with rabbit anti-ghrelin RY1601 was reduced with 1 µM peptide and was abolished with 10 µM and 100 µM. For all secondary antisera used, sections that were incubated without primary antibodies were used to investigate background staining and autofluorescence. There was no indication of non-specific binding of the secondary antibodies.

- 3 Slides were examined and imaged using an Axio Imager Z1 microscope (Zeiss, Sydney, Australia) and a LSM 800 confocal microscope with Airyscan super-resolution analysis (Zeiss). Tile scans taken with a × 10 objective were used for cell counts; for the density analysis, the number of cells was divided by the total area of the mucosal section that was counted. Tile scans were exported to be analysed off-line using ImageJ software (imagej.nih.gov/ij/). Immunoreactive cells were quantified by counting 100–500 cells in sections from each part of the stomach. This analysis was repeated in tissues from three patients. × 40 and × 63 objectives were used for high-resolution analysis. High-resolution images were exported into CorelDraw (Corel, Ottawa, Canada) for final preparation of figures. For analysis of 3D stacks, the Imaris program 8.4.1 (Bitplane, Oxford Instruments, Abington, UK) was used and image rendering was applied where appropriate. To determine whether the ghrelin cells occur in clumps, three pieces of the fundus from three different patients were analysed using the Delaunay triangulation protocol in ImageJ. Ghrelin cells were circled manually and the Delaunay triangulation for neighbour analysis was applied for the selected region of interest.
- 4 Prism 5.0 (GraphPad Software, San Diego, CA, USA) was used to analyse data and present it as mean ± SEM. Differences were evaluated using two-way ANOVA with the Bonferroni post hoc test. *P*value < 0.05 was taken as significant.