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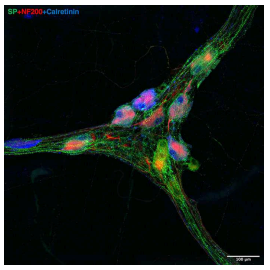
Immunohistochemical labelling of the innervation of dissected human colon wholemounts



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We use this protocol and it's working

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Abstract

This protocol outlines basic methods to localise neurochemical markers in dissected wholemount specimens of human colon using fluorescence immunohistochemical methods

Guidelines

These are a very basic protocol for labelling of nerve cells and their processes in dissected wholemounts of human gastrointestinal tissue.

Materials

- specimen of live human colon (or other gut) tissue
- Krebs solution for preparation containing in (mM): (NaCl; 118mM,KCl; 4.8mM, $CaCl_2$; 2.5mM, $MgSO_4$; 1.2mM, $NaHCO_3$; 25mM, NaH_2PO_4; 1.0mM, glucose; 11mM, bubbled with 95% O_2, 5% CO_2, pH; 7.4)
- Sylgard 184 Elastomer (Dow Corning, Midland MI)
- 4% paraformaldehyde; (Sigma-Aldrich)
- phosphate-buffered saline (PBS) pH 7.4 (137mM NaCl, 10mM phosphate buffer pH7.4)
- Triton X-100; (Sigma-Aldrich)
- glycerol; (Sigma-Aldrich)
- epifluorescence microscope; Olympus IX71 or equivalent with appropriate filters and camera
- Primary and secondary antisera

Troubleshooting

Safety warnings

Before any work on live human tissue, appropriate biosafety requirements must be in place. Consult your institutional biosafety committee for requirements in your jurisdiction

Before start

Before any studies start on live human tissue, formal ethics permits must be in place. See your institutional ethics board for requirements in your jurisdiction.

1 All handling of un-fixed human tissue is exclusively done by staff trained in occupational health and safety requirements for handling hazardous material, wearing appropriate PPE (gloves, gowns and masks) and working in areas designated for human tissue.Users of this protocol should check local requirements with their Institutional Biosafety Committee.

2 Specimens of live human colonic tissue are obtained from operating theatres under the supervision of surgical staff (to avoid interference with needs of pathologists) and placed into room-temperature carbogenated Krebs solution

3 The container is sealed, then placed in a second sealed, watertight container and transported back to the laboratory

4 In the laboratory, in a Microbiological Safety Cabinet, preparations are rinsed repeatedly in fresh oxygenated Krebs solution to flush away contents

5 Preparations are then pinned out mucosal side uppermost in a petri dish lined with 3mm depth of Sylgard 184 Elastomer (Dow Corning, Midland MI) using headless stainless steel insect pins (Australian Entomological Supplies, E184) whilst immersed in fresh carbogenated Krebs solution

6 The mucosa is then removed by sharp dissection and discarded.Where required, submucosa is then removed and transferred to another container. The preparation is then turned over and re-pinned to allow the serosa to be cleared of fat, blood vessels and adhering tissue.

7 The preparation is then re-pinned in a fresh dish in Phosphate-buffered saline (PBS pH7.4) under maximal longitudinal and circular tension.PBS is poured off and replaced with cold 4% paraformaldehyde (4% formaldehyde in 0.1M phosphate buffer, pH 7.2) and the dish is covered and placed in a refrigerator overnight

8 The next day, the preparation is unpinned and placed in fresh 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.2in a sealed container and placed on an orbital mixer at room temperature for 24 hours to ensure full penetration of fixative.

9 All tools, containers and surfaces that have been exposed to unfixed human tissue or contaminating solutions are immersed in 0.1% bleach solution for at least 10 minutes prior to normal cleaning and washing, followed where possible, by wiping with 70% ethanol to remove bleach residues.

10 The specimen is then repeatedly rinsed in PBS for at least 3 × 10 minutes to remove paraformaldehyde

11 The tissue is then pinned in PBS and the circular muscle is removed by sharp dissection, exposing the myenteric plexus.

12 It is then repeatedly rinsed in PBS for at least 3 × 10 minutes

13 Tissue is then immersed in primary antibodies diluted in PBS with 0.3M NaCl, for 24 - 72 hours, then repeatedly rinsed in PBS for at least 3 × 10 minutes

14 Tissue is then immersed in secondary antibodies diluted in hypertonic PBS for 12-24 hours, then repeatedly rinsed in PBS for at least 3 × 10 minutes

15 Tissue is soaked in carbonate/bicarbonate buffered glycerol (pH8.6) and mounted on a slide in the same solution, coverslipped and viewed and photographed on an epifluorescence microscope (Olympus IX71) with appropriate filter sets