Resource Summary Report

Generated by dkNET on Sep 6, 2026

Human colon tissue clearing and Immunohistochemistry

DOI:10.17504/protocols.io.wyeffte
Type: Protocol

Proper Citation

Robert Heuckeroth, Silvia Huerta Lopez, Kahleb Graham, Rajarshi Sengupta 2020. Human colon tissue clearing and Immunohistochemistry. protocols.io dx.doi.org/10.17504/protocols.io.wyeffte

Protocol Information

URL: https://dx.doi.org/10.17504/protocols.io.wyeffte

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Group: Optical Clearing of Tissue

Summary: INTRODUCTION: Bowel pathology is routinely evaluated by sectioning tissue, staining, and light microscopy. Tissue sectioning does not provide robust information about three-dimensional structures. Defining bowel anatomy in three dimensions is especially valuable for understanding human bowel motility disorders. Intrinsic mechanisms controlling bowel motility include the enteric nervous system (ENS), pacemaker cells called interstitial cells of Cajal, smooth muscle cells and PDGFRalpha+ cells. One problem is that the cells that control motility are distributed throughout the bowel wall and the bowel wall is too thick and opaque to permit direct visualization without sectioning.OBJECTIVES: To develop an efficient and reproducible method to make human colon translucent, stain with antibodies, and visualize cells that control bowel motility in three dimensions.METHODS: Tissue is cleaned, trimmed to remove fat, pinned flat, gently stretched and then fixed in 4% paraformaldehyde before storage. To begin clearing tissue is treated with 100% methanol, then permeabilized with Dent's bleach (Methanol, DMSO and hydrogen peroxide) and rinsed with PBS. Blocking is performed for 3 days at room temperature. Incubation with primary antibodies occurs for 14 days at 37 oC. Unbound primary antibody is washed out over the course of one day. Secondary antibody staining is performed for three days at 37 oC. After washing in PBS, tissue is dehydrated using a graded methanol series and then cleared using Murray's Clear (Benzyl Benzoate: Benzyl Alcohol). For confocal imaging, tissue is mounted in Murray's Clear.RESULTS: This approach provides efficient tissue clearing and reproducible staining with a subset of tested antibodies. We routinely obtain images using two or three antibodies and can visualize stained cells all the way through the bowel wall thickness.

Confocal imaging permits excellent visualization and three dimensional reconstruction. We routinely stain 1 cm x 1 cm human colon pieces, but the same methods can be used to stain and image colon tissue many centimeters in length.CONCLUSION: We established an efficient and reproducible method for clearing, staining with antibodies and imaging colon tissue. This approach works well to generate three dimensional images of stained cells. We can visualize many cell types that control bowel motility, but the approach will also probably work to see other cell types with appropriate antibodies. Only a subset of antibodies work with this organic solvent based fixation and clearing method.

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Version: 1

Publication Date: 2020

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Record Creation Time: 20210329T220530+0000

Record Last Update: 20210329T220946+0000

Data and Source Information

Source: