Dissection and fixation of murine colonic tissue for myenteric plexus visualization

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dx.doi.org/10.17504/protocols.io.xz6fp9e

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ABSTRACT

Protocol for harvest of colonic intestinal tissue, with the intent of imaging the myenteric plexus.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS TEXT

Zamboni fixative can be purchased commercially or 1.5% picric acid and 2% paraformaldehyde in 0.1N PBS.
Aquamount mounting media is commercially available

SAFETY WARNINGS

Fixatives can be toxic. Exercise proper use of PPE when handling.

BEFORE STARTING

Prepare Zamboni Fixative

Prepare Krebs-Ringers Solution

1 A ventral midline incision is made and the whole colon is carefully excised into a Sylguard lined dissection dish.

2 Cut along the mesenteric border until the colonic tube is now a rectanular in shape.

3 Pin the colon at 110% of length and width mucosa side up. Gently remove the mucosal layer. Re-pin in a new Sylguard lined dish at 100% ensuring tissue is taught but not stretched.

4 Fix tissue using ice cold Zamboni fixative for 15 minutes.

5 Remove tissue from dish and wash 3 times in PBS for 15 minutes each wash.

6 Cut tissue into 3 sections; oral, middle and anal (in order to fit on slide) and mount on glass slide using Aquamount mounting media and a glass cover slip.

7 Image sample.

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