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Dissection and fixation of murine colonic tissue for myenteric plexus visualization

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

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Abstract

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

Materials

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 start

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.