Dissection and fixation of murine colonic tissue for myenteric plexus visualization

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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 INSTRUCTIONS
Prepare Zamboni Fixative
Prepare Krebs-Ringers Solution

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

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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 rectangular in shape.

3 Pin the colon at 110% of length and width mucosa side up. Gently remove the mucosal layer. Repin 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.