Whole mount dissection and staining of enteric nervous system

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

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ABSTRACT

This protocol details whole mount dissection and staining of enteric nervous system.

ATTACHMENTS

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MATERIALS

Reagents

- Paraformaldehyde (cat# P6148, Sigma-Aldrich)
- Triton X-100 (cat# X100, Sigma-Aldrich)
- SYLGARD^{TM} 184 Silicone Elastomer Kit (cat# 04019862, Dow)
- Hanks' Balanced Salt Solution (HBSS) (cat# 2639065, ThermoFisher Scientific)

Solution

Blocking solution:

- 1X PBS
- 0.1% NaN_3
- 10% FBS
- 0.5% Triton X-100

Paraformaldehyde **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P6148**

Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #X100**

SYLGARD™ 184 Silicone Elastomer Kit **Dow Corning Catalog #04019862**

Perfusion

7m

1 Administer sodium pentobarbital through intraperitoneal injection.

2 Place mouse back in the cage long enough for anesthesia to take effect. Apply a hard toe pinch until mouse no longer reacts, ensuring that the mouse can no longer feel pain before proceeding.

3 Place mouse, abdomen-up, on Styrofoam block. Spray mouse abdomen with 70% ethanol. Grasp skin below ribcage with forceps and cut skin with scissors from middle up either side towards the armpits, cutting through ribcage. Diaphragm should carefully be cut circumferentially.

4 Remove pericardium and peripheral fat to expose heart.

5 Insert the needle into the left ventricle and secure it with vascular clamp. Make a small incision on the right atrium using fine scissors.

6 Start the saline perfusion for 00:07:00 at a constant speed of ~ 1 mL /10 s. 7m

Tissue collection 32m

7 Following perfusion, open the abdomen of the mouse, remove and collect the stomach and duodenum.

8 Place the organs On ice in a tube containing HBSS solution.

9 Open the stomach by cutting on the lesser curvature and open the duodenum along the mesentery line.

10 Wash the tissue in HBSS solution in a petri dish to clean and remove the food.

11 Place the tissue in a Petri dish coated with Sylgard and orient it mucosa up.

12 Grasp the right and left edges of the tissue and pin with 0.20 mm pins.

Note

The tissues should be stretched.

13 Fix the tissue in 4% PFA Overnight at 4 °C in the Petri dish coated with Sylgard. 7m

14 Wash in PBS.

14.1 Wash in PBS for 00:05:00. (1/5) 5m

14.2 Wash in PBS for 00:05:00. (2/5) 5m

14.3 Wash in PBS for 00:05:00. (3/5) 5m

14.4 Wash in PBS for 00:05:00. (4/5) 5m

14.5 Wash in PBS for 00:05:00. (5/5) 5m

15 Unpin the tissue and keep it stored at 4 °C in 1X PBS containing 0.1% Sodium Azide (NaN_3) before dissecting.

Microdissection of longitudinal muscle and myenteric plexus

16 Add PBS 1X to a Petri dish coated with Sylgard, place the tissue on it and orient tissue with the mucosal layer facing up. Grasp the right and left edges of the tissue and pin them.

Note

The tissues have to be stretched.

17 Under a stereo microscope, scratch the mucosa with the back of curved forceps.

18 Using forceps with fine tips, remove the mucosal and submucosal layer until you expose the circular muscle.

19 Peel away the circular muscle with fine forceps to uncover the myenteric plexus.

20 With a micro-scissor, cut small segments of the dissected tissue containing the longitudinal muscle and the myenteric plexus (LMMP).

21 Store the LMMP preparation at 4 °C in 1X PBS containing 0.1% Sodium Azide (NaN_3) until performing immunofluorescence.

Immunofluorescence staining on whole mount tissues

8h 50m

22 In a 96-well plate, add 200 µL of blocking solution containing 0.1% PBS/NaN_3, 10% FBS and 0.5% Triton X-100 per well needed for the number of tissues. Using fine forceps, transfer each tissue into a separate well with the blocking solution and incubate for 02:00:00 at Room temperature on a shaker. 2h

23 Dilute primary antibodies in the blocking solution.

24 Add 200 µL of primary antibodies solution in new empty wells, transfer each tissue in separate wells and incubate with the primary antibodies Overnight at 4 °C on a shaker. 2h

25 Wash in PBS 1X (by adding 200 µL of PBS 1X in new wells and transferring each tissue separately).

25.1 Wash in PBS for 00:05:00. (1/5) 5m

25.2 Wash in PBS for 00:05:00. (2/5) 5m

25.3 Wash in PBS for 00:05:00. (3/5) 5m

25.4 Wash in PBS for 00:05:00. (4/5) 5m

25.5 Wash in PBS for 00:05:00. (5/5) 5m

26 Dilute secondary antibodies at 1:500 in the blocking buffer.

27 Add 200 µL of secondary antibodies solution in new empty wells, transfer each tissue in separate wells and incubate for 02:00:00 at Room temperature on a shaker. 2h

28 Wash in PBS 1X (by adding 200 µL of PBS 1X in new wells and transferring each tissue separately).

28.1 Wash in PBS for 00:05:00. (1/5) 5m

28.2 Wash in PBS for 00:05:00. (2/5) 5m

28.3 Wash in PBS for 00:05:00. (3/5) 5m

28.4 Wash in PBS for 00:05:00. (4/5) 5m

28.5 Wash in PBS for 00:05:00. (5/5) 5m

29 Place the tissue on the slide and mount it between slide and coverslip with prolong gold w/ DAPI.

30 Let it dry Overnight at Room temperature in a slide folder. 2h