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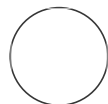
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Protocol status: Working
We use this protocol and it's working

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Whole mount dissection and staining of enteric nervous system

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ABSTRACT

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

ATTACHMENTS[842-2179.docx](#)

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MATERIALS

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Reagents

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

Solution

Blocking solution:

- 1X PBS
- 0.1% NaN₃
- 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 $^{\circ}\text{C}$ 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 μ L 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 μ L 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

