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Mouse perfusion

Forked from Mouse perfusion

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

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Disclaimer

The **protocols.io** team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Abstract

General protocol for mouse perfusion fixation. In this version, other tissues outside of the brain are fixed and ready for processing.

Materials

DPBS
pentobarbital sodium and phenytoin sodium
4% paraformaldehyde (diluted from
Pierce™ 16% Formaldehyde (w/v), Methanol-free **Thermo Fisher Catalog #**28908) in 1X DPBS

Troubleshooting

Mouse Perfusion

2d 0h 10m

1 Anesthetize mouse using pentobarbital sodium and phenytoin sodium. A 30g mouse would need roughly 100 ul of this pentobarbital sodium and phenytoin sodium solution.

2 Ensure deep anesthesia with paw pinch. Mouse should not respond.

3 Using four 26g needles, secure mouse paws to styrofoam.

4 Make an incision along the abdomen, extending up to the rib cage.

5 Expose chest cavity and secure with hemostats clamped to the xiphoid process.

6 Insert 22g needle into the left ventricle and hold via hemostat.

7 Make small incision in the right ventricle.

8 Immediately start peristaltic pump connected to sterile filtered DPBS, flowing at a rate of 10 mL/min

9 Once the perfusate runs clear, stop pump, switch to 4% PFA solution, and resume flow.

10 Perfuse mouse with approximately 100mL of 4% PFA.

11 Remove head with scissors, and carefully dissect out the brain. Remove the skin and limbs and place the skinless, headless carcass into 50mL 4% PFA solution, overnight, at 4C.

12 Scull should be snipped at the base, and peeled away carefully. Care should be taken not to damage underlying brain tissues or olfactory bulb.

13 Place dissected brain in 20mL 4% PFA solution, overnight, at 4C.

1d

14 Wash the tissue with DPBS for a minimum of 3 times, 10 minutes per each wash. 10m

15 Place the gut into DPBS + azide until ready for sectioning

16 Place the brain in 15% sucrose DPBS solution overnight. 1d

17 Once brain sinks, Place in 30% sucrose DPBS solution at 4C until sectioning.