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Mouse perfusion

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Protocol status: Working

We use this protocol and it's working

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Abstract

General protocol for mouse perfusion fixation.

Troubleshooting

- 1 Anesthetize mouse using mixture of ketamine and xylazine. (Ketamine = 100mg ketamine / kg bodyweight, 10mg xylazine / kg bodyweight)
- 2 Ensure deep anesthesia with paw pinch. Mouse should not respond.
- 3 Using four 26g needles, secure mouse paws to corkboard.
- 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 secure with hemostats.
- 7 Make small incision in the right ventricle.
- 8 immediately start peristaltic pump, flowing at a rate of ~8-10mL per min.
- 9 Once the perfusate runs clear, stop pump, switch to 4% PFA solution, and resume flow.
- 10 Perfuse mouse with approximately 50-100mL of 4% PFA. 
- 11 Remove head with scissors, and carefully dissect out the brain. 
- 12 Skull 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.



14 Place in 15% sucrose PBS solution.



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



16 0.05% sodium azide can be included in sucrose solutions to prevent bacterial growth.

