May 14, 2024

Mouse perfusion

DOI
dx.doi.org/10.17504/protocols.io.eq2lyw77pvx9/v1

Bryan Killinger[1]

[1]Rush University

Killinger

Bryan Killinger
Rush University

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.eq2lyw77pvx9/v1

Protocol Citation: Bryan Killinger 2024. Mouse perfusion . **protocols.io** https://dx.doi.org/10.17504/protocols.io.eq2lyw77pvx9/v1

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

Created: May 14, 2024

Last Modified: May 15, 2024

Protocol Integer ID: 99771

Funders Acknowledgement:
MIchael J. Fox Foundation
Grant ID: ASAP-021030
NINDS
Grant ID: 1R01NS128467

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.

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 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.

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.