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Transcardiac Perfusion of Mouse for Brain Tissue

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Maryana Nissan[1], divya.darwinarulseeli[1]

[1]Northwestern University, Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815

Maryana Nissan
Northwestern University

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

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Disclaimer

Abstract

Transcardiac perfusion is a method used for clearing blood and preserving the mouse brain for immunostaining.

Troubleshooting

1 Add Neutralizing Agent to break down Paraformaldehyde [PFA] for proper disposal. We use HydeAway [CAT #2201 from Decon Labs]

2 Turn on the perfusion apparatus and place the tube into 1X Phosphate-Buffered Saline [PBS]

3 Wash the system for 1 round, until the tube is saturated with 1X PBS

4 Saturate anesthetic chamber with Isoflurane

5 Place the mouse in the anesthetic chamber for about 1 minute

6 Remove the mouse from the anesthetic chamber and check reflexes by pinching the foot

7 If the reflex is active, place the mouse back into the chamber. If not, proceed with the perfusion

8 Grip the skin on the chest with forceps, and make an incision beneath the ribcage to expose the diaphragm and liver

9 Carefully make incisions along the diaphragm to expose the heart

10 Tighten the skin with hemostatic forceps, twist, and retract back to expose the heart

11 Secure the heart with forceps with minimal pressure, insert a needle about a depth of 1.5 mm into the left ventricle

12 Puncture the aorta, causing dark venous blood to flow out immediately

13 Wait until the blood gets cleared and lighter in color, until you notice white drops

14 Switch out the tube from 1X PBS into 4% PFA

15 4% PFA will cause the body to become stiff

16 Allow 100mL of 4% PFA to circulate the body

17 Stop circulation and remove the needle from the heart