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Transcardiac Perfusion of Mouse for Brain Tissue

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We use this protocol and it's working

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Abstract

Transcadiac 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