



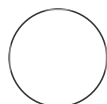
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# Transcardiac Perfusion of Mouse for Brain Tissue

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## ABSTRACT

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

## OPEN ACCESS

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

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

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

- 3 Wash system for 1 round, until the tube is saturated with 1X PBS
- 4 Saturate anesthetic chamber with Isoflurane
- 5 Place mouse in anesthetic chamber for about 1 minute
- 6 Remove mouse from anesthetic chamber and check reflexes by pinching foot
- 7 If reflex is active, place mouse back into chamber. If not, proceed
- 8 Grip the skin on the chest with forceps, 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 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 needle from the heart