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Standard Operating Procedure: Mouse transcardiac perfusion protocol

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ABSTRACT

This protocol details the procedure of the mouse transcardiac perfusion.

ATTACHMENTS

[h78dbu5cf.docx](#)

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KEYWORDS

Transcardiac perfusion, Skin incisions, Heart

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MATERIALS TEXT

Solutions to prepare:

- Avertin (1x)
- Ice cold PBS (1x)
- Ice cold 4% paraformaldehyde
- Ice bucket with ice

Dissection and perfusion tools:

- Peristaltic pump
- Tubing
- Barbed forceps
- Large scissors
- Small scissors
- 1 curved clamp for descending aorta
- Small bone cutter
- Curved fine forceps
- Glass vials
- Tape for labeling
- 22G needles for pinning down paws
- 27G needles for IP injection
- 1ml syringe

BEFORE STARTING

- Wear PPEs before entering perfusion room, i.e. gloves, coat, hair net, safety glasses and face mask.
- Make sure the Hazardous Waste container is not full. Replace if full.
- Turn lever ON before using down draft necropsy table.

Preparing Apparatus and Anesthesia:


- Install the tubes into peristaltic pump. Place one end of the tubing into containers of

ice-cold PBS (1x) and 4% PFA.

- Use parafilm to cover PFA bottle to reduce evaporation.
- Prime the pump by first allowing the fixative to pass the t-stop and follow with PBS (PBS enters the circulation system first). Make sure no air bubble in lines.
- Weight the mouse to the nearest 0.1 gram. Anesthetize with Avertin (dosage: 0.3ml/10g body weight, i.p.). Place the mouse back to the home cage.
- Use toe pinch-response method to determine depth of anesthesia.
- Place the animals on a polystyrene foam lying on the back with face upward, and gently pin the forepaws and hindpaws using 22G needles.

Perfusion Surgery

10m

- 1 Make an incision through the abdominal skin.
- 2 Make two additional skin incisions from the xiphoid process along the base of the ventral ribcage laterally.
- 3 Gently reflect the two flaps of skin to expose thoracic field completely.
- 4 Grasp the cartilage of the xiphoid process with blunt forceps and raise it slightly to insert pointed scissors.
 - 4.1 Cut the thoracic musculature and ribcage between the breastbone and medial rib insertion points and extend the incision rostrally to the level of the clavicles.
- 5 Separate the diaphragm from the chest wall on both sides with scissor cuts.
- 6  Clamp or pin the reflected ribcage laterally to expose the heart.

Optional: Clamp the descending aorta, just below the liver, using the hemostat clamp.

7 Gently grasp the pericardial sac with fingers or blunt forceps, and tear it fully.

8



Secure the beating heart with fingers or blunt forceps, and immediately insert a blunt 25G syringe needle.

Optional: Clamp the needle to the left ventricle using a hemostat.

9 Cut the right atrium with scissors, and at the first sign of blood flow, begin the infusion of 1x PBS at **7.5 ml/min** .

10 Continue perfusion with PBS until the fluid exiting the right atrium is entirely clear, approximately **00:05:00** . 5m

11 Switch perfusate to fixative (4% PFA).

12 Continue PFA perfusion at **7.5 ml/min** for additional **00:05:00** . 5m

Dissection:

13 Decapitate the mouse with large surgical scissors.

14 Make a midline incision to expose the skull.

15 Trim off the remaining neck muscle so that the base of the skull is exposed.

Note: Remove any residual muscle using scissors or rongeurs.

16 Use the sharp surgical scissors to cut through the midline of skull.

17 Gently peel off the skull using blunt forceps and remove the brain out of skull, cut optic nerves if necessary.

18 

Place the brain into a glass vial filled with **10 mL - 15 mL** 4% PFA.

Post-fixation and storage:

2d 0h 5m

19 Keep the brain in fixative for **24:00:00** at **4 °C**.

1d

20 

After 24 hours, rinse the brain with 1x PBS.

20.1 Rinse the brain with 1x PBS 3 times at **00:05:00** intervals, and swirl occasionally (1/3).

5m

20.2 Rinse the brain with 1x PBS 3 times at **00:05:00** intervals, and swirl occasionally (2/3).

5m

20.3 Rinse the brain with 1x PBS 3 times at **00:05:00** intervals, and swirl occasionally (3/3).

5m

21 Store the brains in PBS and keep at **4 °C**.