

## Introduction

For many purposes adequate fixation is obtained by simple immersion of small tissue pieces into the fixative solution. This is the only mode of fixation possible for many tissues. However, a more rapid and uniform fixation is usually obtained if the fixative solution is perfused via vascular system, either through the heart or through the abdominal aorta. The following procedures provide fixation of most rat organs with 4% paraformaldehyde.

## Materials

- Anaesthetic
- Scissors, forceps, and clamps for surgical procedures
- Small forceps with fine claws
- Scalpel
- Vials (5-10ml) with lids for specimens
- 0.9% saline
- 500ml beakers
- 4% paraformaldehyde, fixating solution
- Gloves, eye goggles
- Perfusion pump (or flask with fixative placed upside down about 150cm above the operating table)
- Short syringe needle for heart perfusion of aorta, length about 50mm, outer diameter 1.3-1.5mm
- Perfusion set with drip chamber as used for intravenous blood infusions

## A) Perfusion fixation through the heart

1. Set up perfusion pump, attach perfusion set and perfusion needle. First, run about 100 ml of normal tap water through the tubing to remove any residue. Then place open end of perfusion tube in beaker filled with cold 4% paraformaldehyde (in ice box). The volume of solution should be scaled to size of animal and usually 200-300ml will be sufficient for one animal. Open valve and adjust to a slow steady drip (20 ml/min), and then close valve.
2. Set up surgery site with scissors, forceps and clamps. Give an appropriate amount of anesthetic to the animal. Once the animal is under anesthesia, place it on the operating table with its back down. You may use some tape to hold the appendages so that the animal is securely fixed.
3. Use pinch-response method to determine depth of anesthesia. Animal must be unresponsive before proceeding with the following steps.
4. Make incision with scalpel through abdomen the length of the diaphragm. With sharp scissors, cut through the connective tissue at the bottom of diaphragm to allow access to rib cage.
5. With large scissors, blunt side down, cut through ribs just left of the rib cage midline.
6. Make one center or two end horizontal cuts through the rib cage, and open up thoracic cavity. Clamp open to expose heart and provide drainage for blood and fluids.
7. While holding heart steady with forceps (it should still be beating), insert needle directly into protrusion of left ventricle to extend straight up about 5 mm. **Be careful not to extend the needle too far in, as it can pierce an interior wall and compromise circulation of solutions!** Secure needle position by clamping in place near the point of entry. Release valve to allow slow, steady flow of around 20 ml/min of 0.9% saline solution.
8. Make cut in atrium with sharp scissors, and make sure solution is flowing freely. If fluid is not flowing freely or is coming from animal's nostrils or mouth, reposition the needle.
9. When blood has been cleared from body, change to 4% paraformaldehyde solution (200-300ml).

Take care not to introduce air bubbles while transferring from one solution to the other. It is best to wear protective eye goggles during the whole perfusion process, as sometimes the connecting tubes might come undone and spurt fixating solution into your eyes!



Perfusion is almost complete when spontaneous movement (formalin dance) and lightened colour of the liver are observed. (Note: In general, an adult rat would require around 30-60 mins of perfusion time but this may vary depending on the size of the animal and technique.)

10. Stop the perfusion and excise the tissue of interest. Place them in vials containing the same fixation and fix for another 2 more hours on ice or at 4°C before proceeding to dehydration and embedding. For better results, immersion-fix overnight at 4°C.

## B) Perfusion fixation through the abdominal aorta

1. Prepare materials and animal as stated above (steps 1-3).
2. Open the abdominal cavity by a long midline incision with lateral extension, and move the intestines gently to the left side of the animal.
3. Carefully exposure the aorta below the origin of the renal arteries and very gently free the aorta from overlaying adipose and connective tissues.
4. Hold the wall of the aorta firmly with fine forceps with claws about 0.5-1.0 cm from its distal bifurcation. Insert a bent needle close to the forceps toward the heart into the lumen of the aorta.
5. In a very rapid succession:
  - a. Cut a hole in the inferior caval vein with fine scissors.
  - b. Start the perfusion, and;
  - c. Clamp the aorta below the diaphragm, but above the origin of the renal arteries.

*When performing these manipulations accuracy and speed are essential and the fixation procedure is preferable carried out by two persons. It is particularly important to clamp the aorta rapidly after the perfusion has been started. This is most easily done by compressing the aorta toward the posterior wall of the peritoneal cavity with a finger (wear gloves) which is then replaced by a clamp. Finally, cut the aorta above the compression.*



6. The kidney surface must be blanch immediately and show a uniform, pale color. The flow rate should be at least 60-100 ml/min for an adult rat. Perfuse for 3 min. Stop the perfusion and excise and trim the tissues. Store the tissue in vials and immersion-fix in the same fixative (post fixation step) for 2 hr on ice or at 4°C. For better results, immersion-fix overnight at 4°C.
7. Then, the tissue is now ready for dehydration and embedding.