

**Arizona State University**  
**Institutional Animal Care and Use Committee**  
**STANDARD INSTITUTIONAL GUIDELINE**

**WHOLE ANIMAL PERFUSION**

Perfusion removes blood from organs of interest while rapidly and uniformly preserving tissues for further assessment. While this method can be used for most species including mice, rats, cats, and non-human primates, it is important to note that the specific techniques are dependent on the species, the tissue to be fixed, and how the tissue will be processed following fixation.

**Description of the procedure:**

- 1) If using a perfusion system, make sure there are no bubbles in the line before starting. If using syringes, make sure to have plenty of syringes prefilled with the appropriate solution. The perfusion is started with normal or phosphate buffered saline and may be followed by a fixative, such as formalin, as indicated by the research need.
- 2) The animal is deeply anesthetized as described in the IACUC protocol. Adequate anesthetic depth is confirmed using a firm toe pinch. If the animal reacts, additional time or anesthetic is given.
  - a. The anesthetic agent used needs to ensure the animal maintains appropriate anesthetic depth (i.e., unresponsive to firm toe pinch) during the entirety of the procedure.
    - i. Injectable anesthetic agents (e.g., ketamine-xylazine, avertin) are preferred because the effective anesthesia duration from these agents exceeds the duration of the procedure.
    - ii. Inhalant anesthetic agents (e.g., isoflurane) can be used if the animal is maintained under anesthesia using a nose cone during the entire procedure.
- 3) The following steps need to be performed quickly to ensure the heart is still beating during perfusion.
- 4) Once the animal has reached a surgical plane of anesthesia, grasp the xiphoid process to retract it away from the liver. Using blunt scissors, make an incision caudal to the xiphoid process. Extend the incision laterally along the rib cage.
- 5) Separate the liver from the diaphragm, being careful not to damage it.
- 6) Using blunt scissors, make an incision in the diaphragm along the entire length of the rib cage to expose the pleural cavity.
- 7) Cut the ribs on both sides of the animal to the level of the collar bone.
- 8) Clamp the tip of the sternum with a hemostat and place the hemostat over the head to retract the rib cage. Cut any tissue connecting the rib cage to the heart. When done properly, the thymus lifts away from the heart along with the sternum, providing a clear view of the major vessels.

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- 9) Make a small incision in the right atrium using sharp scissors.
- 10) An appropriate gauge needle (size dependent on species) is passed through the left ventricle into the ascending aorta, but not up to the aortic arch where the brachial and carotid arteries diverge.
- 11) Start infusing the solution through the syringe or perfusion pump. Care should be taken to perfuse slowly to avoid unnecessary rupture of capillaries. The speed will be determined by the size of the animal or specific research parameters.
- 12) If the fluid does not flow adequately, the needle may be abutted against the heart wall. In such cases, adjust the angle of the needle to achieve an appropriate flow.
- 13) Continue the perfusion until the liver is uniformly pale, switching to a different perfusion injectant as needed and described in the IACUC protocol.

**Detailed reference for rodent perfusion including an informative video:**

<http://www.jove.com/video/3564/whole-animal-perfusion-fixation-for-rodents>