



Cornell University
Cornell Center for Animal Resources and Education

CARE101.01 Rodent Anesthesia

The intent of this standard operating procedure (SOP) is to describe methods which have been approved by Cornell's Institutional Animal Care and Use Committee (IACUC) and by the Cornell Center for Animal Resources and Education (CARE). This SOP is intended for use by researchers and CARE staff that anesthetize rodents. Any exemptions to this SOP must be approved by the IACUC prior to their application.

TABLE OF CONTENTS

1. Mouse Anesthesia
2. Rat Anesthesia
3. Rodent Neonates
4. References

1. Mouse Anesthesia

a. Isoflurane

Note: Isoflurane is the method of choice for rodent anesthesia. Gas waste must be scavenged properly, see [CARE SOP 712](#).

- i. Place the animal in the induction chamber.
- ii. Adjust the flowmeter to 0.8–1.5 L/min.
- iii. Adjust the isoflurane vaporizer to 2%–3%.
- iv. For maintenance, use the mask connected to the Bain circuit, and adjust the flowmeter to 400–800 mL/min.
- v. Prevent heat loss until the animal recovers.

Due to their small size, heat loss is rapid in anesthetized rodents. They can be kept warm by covering them with a gauze or a paper towel, or by providing a heat source.

b. Xylazine–Ketamine

- i. In a sterile 10 mL bottle with a rubber stopper, mix 1 mL of ketamine (100 mg/mL) + 0.1 mL of xylazine (100 mg/mL) + 8.9 mL of sterile water for injection. SHAKE WELL BEFORE USE.
- ii. Write on the bottle:
 - "XYLAZINE–KETAMINE FOR MOUSE: 0.1 mL/10 g"

- your initials
 - the expiration date (selecting the earliest expiration date between ketamine, xylazine, or water, for a maximum of 3 months)
 - iii. Keep away from light, in a cool place
 - iv. Inject 0.1 mL/10 g IP.
 - v. Repeat when necessary, half a dose at a time (approximately every 30 minutes).
 - vi. Prevent heat loss until the animal recovers.
- c. Avertin

Note: Proper storage of Avertin is critical to prevent the formation of irritating degradation products.

- i. Components
 - 2, 2, 2-Tribromoethanol
 - Tertiary amyl alcohol
- ii. Concentrated solution
 - Prepare 25 g Tribromoethanol in 15.5 mL amyl alcohol.
 - Write on the bottle:
 - a. "50X Avertin"
 - b. your initials
 - c. Write the expiration date (1 year).
 - Store in freezer, away from light.
- iii. Solution for injection
 - Mix 1 mL of the concentrated solution in 50 mL of 0.9% sterile saline for injection, in a sterile container.
 - Heat the solution to 40 °C, maintaining sterility.
 - Shake well.
 - Identify the container and store in refrigerator for a maximum of 6 months.
- iv. Dose
 - 0.15 mL/10 g IP.
- v. Warning
 - Prevent heat loss until the animal recovers.
 - If avertin is not properly stored, degradation products may cause severe peritonitis.

2. Rat Anesthesia

- a. Isoflurane
 - i. Place the animal in the induction chamber.
 - ii. Adjust the flowmeter to 0.8–1.5 L/min.
 - iii. Adjust the isoflurane vaporizer to 2%–3%.
 - iv. For maintenance, use the mask connected to the Bain circuit, and adjust the flowmeter to 400–800 mL/min.
 - v. Prevent heat loss until the animal recovers.

b. Xylazine–Ketamine

i. Recipe A

- In a sterile 10 mL bottle with a rubber stopper, mix 8.75 mL of Ketamine (100 mg/mL), and 1.25 mL of Xylazine (100 mg/mL). SHAKE WELL BEFORE USE.
- Write on the bottle:
 - a. "XYLAZINE–KETAMINE FOR RAT: 0.05–0.10 mL/100 g IP"
 - b. your initials
 - c. expiration date (selecting the earliest expiration date between ketamine, xylazine, or water, for a maximum of 3 months.)
- Keep away from light, in a cool place.
- Administer 0.05–0.10 mL/100 g IP.
- Repeat as required with 1/3 to 1/2 dose at a time (approximately every 30 minutes).
- Prevent heat loss until the animal recovers.

ii. Recipe B

- In a sterile 10 mL bottle with a rubber stopper, mix 3.75 mL of Ketamine (100 mg/mL), 0.5 mL of Xylazine (100 mg/mL), and 5.75 mL of sterile water for injection. SHAKE WELL BEFORE USE.
- Write on the bottle:
 - a. "XYLAZINE–KETAMINE FOR RAT: 0.2 mL/100 g IP"
 - b. your initials
 - c. expiration date (selecting the earliest expiration date between ketamine, xylazine, or water, for a maximum of 3 months.)
- Keep away from light, in a cool place.
- Administer 0.2 mL/100 g IP.

iii. Repeat as needed with 1/3 to 1/2 dose at a time (approx. every 30 minutes).

iv. Prevent heat loss until the animal recovers.

3. Rodent Neonates

Important: This technique may only be used in neonatal altricial (hairless) rodents, generally <10 days old.

- a. Place the neonate on a latex covered bed of crushed ice, or place in the cut off finger of latex glove and immerse in ice water.
- b. Alternatively, place the neonate inside a glass test tube lined with paper and immerse in ice water.

Note: The time of onset of lethargy may be considerably slower than in the other methods.

- c. Monitor the readiness of the animal for a procedure by noting lethargy and immobility. Expect the neonate to remain immobile for up to 10 minutes. If additional time is needed for the procedure, maintain immobility by keeping the neonate on a latex covered cold pack.
- d. Illuminate the surgical field by use of a fiber optic light source, as incandescent bulbs may cause inadvertent and uncontrollable warming.
- e. Recover pups and slowly re-warm them in an incubator at 33°C or in a warm nest. Complete recovery typically requires **30–60** minutes.

4. References

- a. Flecknell, P., *Laboratory Animal Anesthesia*; Academic Press, New York; 1996.
- b. Kohn, D.F., Benson, G.J., Wixson, S.K., White, W.J., *Anesthesia and Analgesia in Laboratory Animals*; Academic Press, New York, 1997; Chapter 15.
- c. Danneman, P.J., Mandrell, T.D. Evaluation of five agents/methods for anesthesia of neonatal rats. *Lab. Anim. Sci.* 1997 Aug;47(4):386-95.

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