1. PURPOSE

This Standard Operating Procedure (SOP) describes methods for anesthetizing rodents.

2. RESPONSIBILITY

Principal investigator (PI) and their research staff.

3. GENERAL CONSIDERATIONS

3.1. Do not fast rodents before anesthesia due to their inability to vomit.
3.2. Rodents can be anesthetized with either inhalants or injectable anesthetic drugs. The use of inhalant anesthetic is the method of choice whenever possible.
3.3. Heat loss is rapid in anesthetized rodents. Keep the animals warm by providing a heat source until the animal has recovered from anesthesia. Care should be taken not to overheat or burn the animal; do not place animals directly in contact with the heat source; use a drape or other material as a barrier.
3.4. Never leave an anesthetized animal unattended. Monitor anesthetized animals until they can right themselves, ambulate, and move in their cage.

4. MATERIALS

4.1. Material or equipment to provide or conserve body heat: heating pack, infra-red warming pad. Do not use electric heating pads unless specifically designed with laboratory rodents.
4.2. Ophthalmic ointment (natural tears)
4.3. Gas anesthesia machine
4.4. Induction chamber constructed of a see-through material (polypropylene, etc.)
4.5. Rodent anesthesia nosecone or mask
4.6. Isoflurane
4.7. Ketamine (100 mg/mL) *Controlled Drug
4.8. Xylazine (20 mg/mL)
4.9. Sterile isotonic saline (0.9% saline)
4.10. Crushed ice or ice pack
5. PROCEDURES FOR ADULT RODENT

5.1. Isoflurane anesthesia (see more details; SOMOFLO (Kent Scientific) or analog machine instructions):

5.1.1 Induction:

5.1.1.1. Connect the isoflurane bottle to the adaptor of the SOMOFLO machine or fill the vaporizer reservoir with isoflurane.
5.1.1.2. Open Oxygen source (valve is placed on the wall, be sure not to exceed 10PSI)
5.1.1.3. Turn on the SOMOFLO machine (allow the automatic machine priming)
5.1.1.4. Adjust the air/oxygen flowmeter to 500ml/min, isoflurane vaporizer (2-5%) – High Flow.
5.1.1.5. Let the machine run for 3-5 min at High Flow to allow the distribution of the isoflurane.
5.1.1.6. Place the animal in the induction chamber
5.1.1.7. Adjust the isoflurane vaporizer between 2% to 5%.

5.1.2 Maintenance:

5.1.2.4. Remove the animal from the induction chamber and use a nosecone or mask connected to the Bain circuit.
5.1.2.5. Adjust the flowmeter to 200ml/min isoflurane vaporizer to 1.5 to 2.5% - Low Flow.
5.1.2.6. Apply ophthalmic ointment (natural tears) to both eyes to prevent dryness and damage to the cornea. Reapply as needed.
5.1.2.7. Continuously monitor the animal during anesthesia and adjust the level of isoflurane as needed according to monitored parameters:
   5.1.2.7.1. Presence of reflexes/response to stimuli (pedal withdrawal reflex)
   5.1.2.7.2. Respiratory rate and breathing pattern
   5.1.2.7.3. Mucous membrane color surrounding the nose and mouth (should remain pink)

5.1.3 Recovery:

5.1.3.1. Keep the animal under close supervision until it starts to recover.
5.1.3.2. Transfer the animal to its cage once it begins to move and allow it to recover fully (sternal position).
5.1.3.3. Provide supplemental heat during the recovery period.

5.2. Ketamine/Xylazine anesthesia:

5.2.1 The injectable anesthetic dose can vary with the sex, age, strain, and body condition of the animal.

5.2.2 Recommended anesthetic dose (Mice): ketamine 100 mg/kg, xylazine 10 mg/kg.
   Recommended anesthetic dose (Rat): ketamine 75 mg/kg, xylazine 5 mg/kg

5.2.3 When working with a new mouse strain, administer 75% of the recommended dose. If pedal withdrawal reflexes remain after 5 minutes, help the remaining 25% of the recommended dose. Shake the solution thoroughly before use.

5.2.4 To prepare the solution, in a sterile vial or bottle with a rubber stopper, mix:

   For Mice:
1 mL of ketamine (100 mg/mL)  
0.5 mL xylazine (20 mg/mL)  
8.5 mL of sterile isotonic saline or sterile water for injection.  

**For Rats**  
3 mL of ketamine (100 mg/mL)  
1 mL xylazine (20 mg/mL)  

5.2.5 Indicate the expiration date on the vial or bottle **(Maximum 3 days)** and the final concentration of the mixture.  
5.2.6 Administer 0.1 mL/10g (mice) and 0.1 mL/100g (Rat) body weight intraperitoneally or subcutaneously for the recommended dose.  
5.2.7 Apply ophthalmic ointment (natural tears) to both eyes to prevent dryness and damage to the cornea. Reapply as needed.  
5.2.8 After 5 minutes, monitor anesthetic depth by verifying the pedal withdrawal reflex.  
5.2.9 The duration of anesthesia is approximately 30 minutes.  
5.2.10 After 30 minutes, a half dose may be administered as needed.  
5.2.11 Provide supplemental heat and monitor until recovery (sternal position).

---

**6. PROCEDURES FOR NEONATAL RODENTS**

6.1. Hypothermia:  
6.1.1 Use only in animals **less than seven days of age.**  
6.1.2 Provides immobilization and mild analgesia for short, minor procedures.  
6.1.3 Protect the pup in a glove or nylon-covered crushed ice to avoid direct contact to the skin.  
6.1.4 Induction: Immerse the pup in covered ice water or crushed ice for **3 to 4 minutes.**  
6.1.5 Maintenance:  
6.1.5.1. Place the pup on a paper-covered ice pack.  
6.1.5.2. Use a fiber optic surgical lamp if necessary, as incandescent lamps will warm the animal and interfere with anesthesia.  
6.1.5.3. The duration of anesthesia is approximately 10 minutes.  
6.1.6 Recovery:  
6.1.6.1. Remove the animal from the ice pack and allow it to warm.  
6.1.6.2. Recovery time can be up to 1 hour.  

6.2. Isoflurane anesthesia:  
6.2.1 Neonates require a higher concentration of Isoflurane than adults (Maintenance at 3-4%).  
(See section 5.1 for detailed procedures).