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### **1. PURPOSE**

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

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### **2. RESPONSIBILITY**

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Principal investigator (PI) and their research staff.

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### **3. GENERAL CONSIDERATIONS**

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- 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.
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### **4. MATERIALS**

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- 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

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## 5. PROCEDURES FOR ADULT RODENT

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### 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**

- 3mL of ketamine (100mg/mL)
- 1mL xylazine (20mg/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 0.1ml/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).

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## 6. PROCEDURES FOR NEONATAL RODENTS

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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).

**SOP 201 RODENT ANESTHESIA**

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Approved by the BGU Animal Policy and Welfare Oversight Committee