

**1. PURPOSE**

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This Standard Operating Procedure (SOP) describes the anesthesia procedure for conducting lung physiology measurements in mice and rats using the Flexivent apparatus.

**2. RESPONSIBILITY**

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Principal investigator (PI) and their research staff and students.

**3. MATERIALS**

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- 3.1. Xylazine (20mg/mL)
- 3.2. Pentobarbital (54.7mg/mL) \*Controlled drug
- 3.3. Lidocaine 2%
- 3.4. Pancuronium bromide (1mg/mL)
- 3.5. Ophthalmic ointment (natural tears)
- 3.6. Tracheal tube
- 3.7. Flexivent®, computer-controlled small animal ventilator

**4. PROCEDURES**

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- 4.1. Weigh each animal individually and calculate the appropriate dose of anesthetics.
- 4.2. Administer intraperitoneally, mixed in the same syringe:
  - 4.2.1. Mouse model:  
xylazine 10 mg/kg + pentobarbital 50 mg/kg
  - 4.2.2. Rat model:  
xylazine 5 mg/kg + pentobarbital 50 mg/kg
- 4.3. Apply ophthalmic ointment (natural tears) to both eyes to prevent dryness and damage to the cornea.
- 4.4. Leave the animals in their home cage for 2-3 minutes.
- 4.5. Verify the absence of pedal reflex by pinching the toes and/or footpads with a pair of forceps.
- 4.6. Once the reflex is completely lost, perform the tracheotomy.
- 4.7. Connect the tracheal tube to the Flexivent.
- 4.8. Administer pancuronium bromide.
- 4.9. Proceed to measurements for up to a maximum of 45 minutes after injection of xylazine-pentobarbital.
- 4.10. Euthanize animal according to SOP.