Purpose

Ketamine is a dissociative anesthetic used so that animals do not experience the pain. Xylazine is a sedative causing muscle relaxation and anesthesia. For Ketamine and Xylazine cocktails there is a range of mixtures that are acceptable and effective. Here we provide a recommended mixture that requires dilution with saline or sterile water and can be premixed in the lab under sterile conditions.

These drugs must be purchased as pharmaceutical grade injectable vials. Note that Ketamine requires a DEA license to purchase and use. An excellent alternative is isoflurane, which is an inhaled drug that can be administered for short term procedures by using the drop method or maintained for longer procedures with the anesthesia machine available in the ARF.

PREPARATION OF KETAMINE/XYLAZINE COCKTAIL FOR MICE

- Use of a sterile injection vial is required (e.g. redtop blood collection tube; commercial injection vial)

- Mixing instructions for 10 mL Total volume and Lower ketamine dosage
  - Verify the concentration of your drugs prior to mixing
  - For a 10mL vial using Ketamine 100 mg/mL and Xylazine 20 mg/mL add:
    - 1.75 mL Ketamine (100 mg/mL)
    - 1.25 mL Xylazine (20 mg/mL)
    - 7 mL saline or sterile water for injection

- Use of the following template for a label is recommended:
  - Mouse Anesthetic Mix: Ketamine/Xylazine
  - Dosage: 0.1 ml/ 20gm IP
  - Delivers: 87.5 mg/kg Ketamine/12.5 mg/kg Xylazine
  - Concentration: 17.5 mg/mL Ketamine/2.5 mg/mL Xylazine
  - Expires: ____________

- The expiration date for the cocktail is determined by whichever of the components expires first
  - E.g.: Ketamine expires 12/10/2016, Xylazine expires 10/10/16 and sterile water for injection expires 1/12/2017; the expiration date for the cocktail is 10/10/16

Alternative Mixing Instructions for 2 mL total volume and Higher Ketamine Dosage:
Policies and Procedures

SECTION: IACUC

CHAPTER: Animal Use Policies

POLICY: Ketamine and Xylazine Anesthesia

• Verify the concentration of your drugs prior to mixing
• For a 2 ml vial using Ketamine 100mg/mL and Xylazine 20 mg/ml add:
  o 0.2 ml of Ketamine (100mg/ml)
  o 0.1 ml of Xylazine (20mg/ml)
  o 1.7 ml of Sterile Saline or Sterile Water for injection

Use of the following template for a label is recommended:

• Mouse Anesthetic Mix: Ketamine/Xylazine
• Dosage 0.1 ml/10 gram mouse IP
• Delivers: 100mg/kg Ketamine/10mg/kg Xylazine
• Concentration: 10mg/ml Ketamine/1 mg/ml Xylazine
• Expires:__________

• The expiration date for the cocktail is determined by whichever of the components expires first
  o E.g.: Ketamine expires 12/10/2016, Xylazine expires 10/10/16 and sterile water for injection expires 1/12/2017; the expiration date for the cocktail is 10/10/1

Completing Section E3 Anesthesia and Sedative (Including Pre-anesthesia) for the IACUC protocol:
**Pharmaceutical grade** | **Agent(s)** | **Dosage & Route** | **Verification of Anesthesia / Additional Information**
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Yes | Ketamine/Xylazine Mixture (0.2 ml of Ketamine 0.1 ml of Xylazine and 1.7 ml of sterile saline) Final concentration in 2 ml mixture is 10 mg/ml ketamine and 1 mg/ml xylazine | 0.1 ml of ketamine/xylazine mixture per 10 g mouse bwt. administered by intraperitoneal (IP) injection (100mg/kg ketamine and 10mg/kg xylazine) | Mice will be examined for lack of response to a foot pinch and regular, rhythmic breathing. Anesthesia can be maintained for 15-20 min. for surgery.

No* | Ketamine/Xylazine Mixture (1.75ml Ketamine 1.25 ml of Xylazine and 7 mL of sterile Saline) Final concentration in 10 ml Mixture is 17.5 mg/mL Ketamine And 2.5mg/ml Xylazine | 0.1 ml of ketamine/Xylazine Mixture per 20 g mouse bwt Administered by intraperitoneal (IP) injection (87.5mg/kg ketamine and 12.5mg/kg xylazine) | Mice will be examined for lack of response to foot pinch and regular, Rhythmic breathing.

X | Ketamine only (diluted in sterile saline) 0.1 ml of ketamine (100 mg/ml) diluted in 1.9 ml of sterile saline (5mg/ml Ketamine final solution) | 0.1 ml of diluted ketamine mixture per 10 g mouse bwt administered by intraperitoneal injection (final dose administered for maintenance of anesthesia is 50 mg/kg bwt) | After surgery, mice will be administered ketamine only as needed to maintain anesthesia. Absence of corneal blink and paw withdraw reflexes will be checked for the verification of anesthesia.

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*Ketamine and Xylazine are used as a combination. Ketamine is also used separately.

*If any controlled substance is to be used, provide the Drug Enforcement Administration (DEA) registration number
and the name of the licensee

If compound is not pharmaceutical grade (off the shelf, undiluted) then fill in section E6 below.

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**Completing Section E6. Non-pharmaceutical compounds used in live animals**

List all compounds from E2-E5 that are **non-pharmaceutical** grade, this includes dilution of pharmaceutical grade drugs. Provide a) scientific rationale for use of each compound in animals, b) source of compound, grade/purity, c) formulation, and d) quality control to include sterility, pyrogenicity, stability, pH, osmolality, pharmacokinetics, physiological compatibility, and quality control. For Multiple agents create additional lines.

**Rationale:** Cocktail is to fully anesthetize the animal, prevent pain and relax muscles.

**Source/Purity:** Ketamine ***source*** (100mg/ml), Xylazine ***** source**** (20 mg/ml)

**Formulation:**

**Quality Control:** Cocktail prepared with a sterile vial and needles. The vial is Vortexed prior to usage and sterile needles are used for withdrawal. The vial is stored in a double locked box at room temperature. Each secondary container is labeled with name of drug, concentration, preparation date and initials, expiration date, and “for animal use only”

Expiration date is same date as the individual drug with the earliest expiration date.