Laboratory Animal Resources Guidelines

**Guidelines for Anesthesia and Analgesia in Mice**

1. **Purpose**- This document has been designed by Indiana University Bloomington Laboratory Animal Resources veterinary staff as a guideline for tranquilization, anesthesia and analgesia of laboratory mice. This is not intended to be an inclusive tutorial on all possible drug combinations that can be used in mice. The following guidelines are also general recommendations and consequently do not include reference to specific research-associated concerns. If you have questions about the use of anesthetics or analgesics for your particular situation, or if you have questions or comments about this document, please contact LAR at x5-2356 or [lar@indiana.edu](mailto:lar@indiana.edu).
2. **Special Concerns in Mouse Anesthesia**

There are several factors that should be considered prior to initiating an experimental procedure that requires anesthesia in the mouse. Newly arrived animals should be **acclimated** to their new surroundings for at least **48 hours** before use. The age of the animals and their body weights should also be taken into account when selecting an anesthetic protocol. Preanesthetic fasting is usually not necessary; however, if fasting is employed is should be limited to no more than 2-3 hours due to the high metabolic rate of small rodents. **Water should never be restricted**.

There are 3 basic methods of anesthetic delivery for rodents: parenteral, inhalation, or a combination of both. **Parenteral anesthesia** may be administered via the intraperitoneal (IP) injection, the animal is held in a head-down position and a 22-27 gauge needle is inserted into the lower right or left abdominal quadrant just off the midline. Subcutaneous (SC) injections can be given by tenting the skin of the back and injecting into the space between the skin and underlying muscle. Intravenous (IV) injections are most often given into the lateral tail veins. These vessels are to be dilated by placing the tail in warm (not hot) water for 1-2 minutes prior to injection or placing the animal on a warm water recirculating pad. Use of a 25 or 27 gauge needle should be adequate for IV injections in mice. Intramuscular (IM) injections are not recommended in mice. Dosing inaccuracies and complications such as tissue irritation, lameness and self-mutilation can result. Training in proper restraint and injection techniques is required for those persons performing these procedures.

may be delivered by a chamber or facemask. Chambers can be made by using a large covered glass, plexiglass or plastic container with anesthetic soaked cotton balls or gauze squares in the bottom. See Table 1 for dose recommendations. The mouse must be prevented from coming into direct contact with the liquid inhalant anesthetic by placing a mesh grid over the cotton/gauze. Flow through anesthesia chambers and facemasks require a gas anesthesia machine with an oxygen source and a precision vaporizer. Due to the small respiratory capacity in mice, a nonrebreathing system should be used. Anesthesia chambers and facemasks are commercially available. For anesthetic events lasting greater than 5 minutes and whenever facemasks are used, an ophthalamic ointment (e.g. Paralube® or Lacrilube®) must be applied to the eyes to prevent corneal drying and trauma.

When using inhalant anesthesia, a fume hood or an anesthetic system equipped with a gas scavenging system should be used to minimize occupational exposure to exiting gases.

1. **Prevention of Hypothermia**

In order to prevent hypothermia during anesthesia, which slows recovery and further stresses the animal, supplemental heat should be provided in the form of a recirculating warm water blanket or isothermal heat source (e.g. Gaymar Stryker Heating/Cooling T/Pump, Snuggle®Safe). Electric heating pads are discouraged because of uneven heating and tendency to cause thermal injury. Regardless of the heat source, the animal must never be placed directly on the heat but should be separated from it by a towel or sterile drape. Covering the animal with a sterile drape also helps to conserve body temperature. When covering an anesthetized animal, be careful not to place excessive pressure over the thorax. See also *Guidelines for Surgery in Rodents*.

1. **Monitoring and Recovery**

Regardless of the anesthetic administered, the mouse must be monitored to avoid excessive depression of cardiac and respiratory functions, or insufficient anesthesia. This is characterized by poor muscle relaxation, movement in response to surgical stimulation or vocalization. Parameters that should be monitored in an anesthetized mouse include anesthetic depth, respiratory rate and pattern (normal undisturbed RR = 180/min., a slow rate drop of 50% is acceptable during anesthesia), mucous membrane color (should be pink not blue or grey). An ophthalamic ointment (Paralube®) must be applied to the eyes of any animals receiving injectable anesthetics or to those animals anesthetized with gas anesthetics for greater than 5 minutes. Depth of anesthesia may be assessed by an inability to remain upright, loss of movement, loss of blink reflex, muscle relaxation and loss of response to reflex stimulation (such as a toe pinch). Movement of the chest wall and observation of abdominal movements may be used to assess respiratory rate. **Because the mouse has a greater body surface area to body mass ratio than larger animals, thermal support is critical to their recovery.** Heat loss may be minimized by placing a warm water blanket drape between the animal and the table or by administering warmed subcutaneous or intraperitoneal fluids during or after surgery (5-10 ml/kg/hr). Fluids such as warmed saline or lactated Ringer’s solution are also important for correcting volume deficits. Nutritional support is also critical following recovery and food can be provided on the cage floor or in the form of nutrient gels available from LAR.

It is highly preferable to recover all animals in separate clean cages without bedding within the room used for surgical preparation the so that they can be continually monitored. If a large number of surgeries are being conducted at one time, animals may be housed together following anesthesia but prior to recovery only if they are continually observed (at least once every 2-3 minutes) by a member of the research lab to assure that more alert rodents are not cannibalizing nonresponsive cage mates. A standard rodent diet should be provided as soon as the animal has recovered sufficiently to move and eat. Moist chow can be used to encourage eating. To prevent injury or cannibalization, anesthetized mice should not be placed back in a cage with other mice until they are fully ambulatory.

1. **Anesthesia**
2. **Preanesthetic Agents and Anesthetic Agents**

Atropine (0.05 mg/kg-0.1 mg/kg SC) may be administered subcutaneously as a preanesthetic agent approximately 15 minutes before anesthesia. Atropine prevents the drop in heart rate and excessive salivation that may be caused by agents such as inhalant anesthetics and ketamine. Glycopyrrolate (0.01-0.02 mg/kg SC) is an effective alternative to atropine when given 15 minutes prior to surgery. These agents are not frequently used for rodent anesthesia.

1. **Preferred Anesthetics**

Isoflurane has become the anesthetic agent of choice for both short and lengthy procedures due to its rapid and reliable recovery. If using a precision vaporizer to deliver anesthetic, the machine must be compatible with the specific inhalant anesthetic. Sevoflurane is another common inhalation anesthetic with a quick recovery time.

The injectable anesthetic combination of choice is Ketamine 80-120 mg/kg IP + xylazine (Rompun®) 5-10 mg/kg IP to produce 30-45 minutes of anesthesia. If one needs more anesthetic, only use 1/3 the original calculated dose of ketamine. Xylazine should not be redosed due to its hypotensive effects.

**Table 1. Inhalant Anesthetics Used in Mice**

|  |  |  |
| --- | --- | --- |
| **Drug** | **Dosage** | **Comments** |
| Isoflurane (Forane®, Aerane®) Recommended | 4-5% for induction  1-2% for maintenance | 300 μl in a 500 ml container- chamber induction for brief anesthesia.  Maintenance requires use of a calibrated vaporizer. |
| Sevoflurane | 5-7% induction  3-4% maintenance | Requires use of a calibrated vaporizer. |

**Table 2. Injectable Anesthetics and Tranquilizers Used in Mice**

|  |  |  |  |
| --- | --- | --- | --- |
| **Drug** | **Dosage & Route** | **Duration of Anesthesia** | **Comments** |
| Barbiturates | | | |
| Thiopental (Pentothal®) | 25-50 mg/kg IV  50 mg/kg IP | 10 min.  Unproven | Dose dependent respiratory depression and hypothermia |
| Pentobarbital (Nembutal®) | 40-50 mg/kg IP sedation  70-85 mg/kg IP anesthesia | 10-300 min. | Respiratory depression/poor analgesia |
| Dissociatives | | | |
| Ketamine (Ketoset®)\* | 100-200 mg/kg IP | Unproven | Poor muscle relaxation/mild analgesia |
| Ketamine + Medetomidine\* | 75 mg/kg K + 1 mg/kg M IP |  | Surgical anesthesia 20-30 min.;  Sleep time 60-120 min. |
| **Ketamine + xylazine (Rompun®)**  **Recommended** | 80-120 mg/kg ketamine IP + 5-10 mg/kg xylazine IP | 30-45 min. | Anesthetic depth varies from sedation to anesthesia |
| Ketamine + xylazine + acepromazine (Triple sedative) | 13 mg/kg ketamine + 0.66 mg/kg xylazine + 0.33 mg/kg ace IP (0.01-0.03 ml/100g) | Unproven | Sedative- not appropriate for anesthesia alone |
| Ketamine + acepromazine | 44 mg/kg ketamine I +0.75 mg/kg ace IP | 20-30 min. | Sedation only |
| Ketamine + diazepam (Valium®) | 100-200 mg/kg ketamine IP + 5 mg/kg dia IP | 20-30 min. | Sedation/immobilization |
| Ketamine + midazolam (Versed®) | 100 mg/kg ket IP +  5 mg/kg mid IP | 20-30 min. | Immobilization |
| Other | | | |
| Urethane | 1000 – 1500 mg/kg IP |  | Caution! Prolonged anesthesia; terminal procedures only; carcinogenic and mutagenic |
| Propofol (Diprivan®) | 12-26 mg/kg IV | 5-7 min. | Titrate as needed |

Subcutaneous (SC), Intraperitoneal (IP), Intravenous (IV)

\*Ketamine alone is not adequate for deep anesthesia or procedures that are painful. It is only to be used for immobilization.

\*Reversal of α2agonists such as xylazine and medetomidine can be accomplished by giving atipamazole (Antisedan®) 1-2.5 mg/kg IM, IP, SC or IV

1. **Analgesia**

Unrelieved pain can have profound negative physiologic consequences, which may alter research results. Mice show a variety of responses to pain, some of which may be fairly subtle and easily missed on casual examination. Pain evaluation in mice consists of evaluating behavioral and physiologic parameters.

Behavioral Signs of Pain Physiologic Indicators of Pain

Reluctance to move Elevated blood pressure

Abnormal posturing Elevated heart rate

Social isolation Elevated respiratory rate

Decreased appetite Changes in body temperature

Vocalization Dilated pupils

Decreased grooming

Aggression

Self-mutilation

Piloerection

Squinted eyes/pale eyes

For short-term management (less than 7 days) of moderate to severe pain, the LAR staff recommends SC injections of buprenorphine (0.05-0.1 mg/kg) 2-3 times per day. A single injection of buprenorphine will typically last 8 hours, but there is considerable variation in duration. The animals should be observed carefully so the optimum dose and frequency can be determined.

Table 3. Analgesics Used in Mice

|  |  |  |
| --- | --- | --- |
| Drug | Dose | Duration |
| Buprenorphine (Buprenex®)a | 0.05-0.1 (2 mg/kg) mg/kg SC or IV | 6-12 hrs. |
| Buprenorphine SR | 1-1.2 mg/kg SC | Lasts for 3 days |
| Carprofen (Rimadyl®) | 5 mg/kg SC or IP, 10 mg/kg PO | 24 hrs. |
| Flunixin (Banamine®) | 2.5 mg/kg SC | 12-24 hrs. |
| Ibuprofen | 7.5-40 mg/kg PO (0.2 mg/ml water) |  |
| Ketoprofen | 5 mg/kg SC | 24 h |
| Meloxicam (Metacam®) | 1-2 mg/kg SC, 5 mg/kg PO | 12-24 hrs |
| Morphine a,b | 2-5 mg/kg SC | 1-4 hrs. |
| Tramadol | 5 mg/kg SC, IP |  |
| Lidocaine 1% | 4 mg/kg (0.4 ml/kg) | 1.5-2 hours |
| Bupivacaine 0.25% | 1-2 mg/kg (0.4-0.8 ml/kg) | 4-12 hours |

Subcutaneous (SC), Intraperitoneal (IP), Intravenous (IV), oral (PO)

a In addition to being an analgesic, this drug also acts as a sedative. If this drug is administered as an animal is recovering from anesthesia, the animal must be observed carefully for cumulative sedative effects of the anesthetics and the analgesics.

b This drug has a broad range of recommended doses. It is recommended that the animal be given the lowest dose in the range and be observed for signs of pain or discomfort. Additional analgesic may be administered if necessary at the next scheduled dosing time.

1. **Local Anesthetics**

Lidocaine and bupivacaine are the two most commonly used local anesthetics. Lidocaine has a rapid onset (1-2 minutes) and short duration (1.5-2.0 hours) of action; bupivacaine has a slower onset (5-10 minutes), but a much longer duration of action (4-12 hours, site dependent). Maximum safe doses for most species are:

**Lidocaine: 4 mg/kg (0.4 ml/kg of a 1% solution)**

**Bupivacaine: 1-2 mg/kg (0.4-0.8 ml/kg of a 0.25% solution)**

These doses can be diluted in sterile saline to provide a larger injection volume. IV administration of lidocaine or bupivacaine can cause cardiovascular effects (e.g., hypotension, dysrhythmias) and central nervous system depression followed by seizures. To avoid these adverse consequences, each animal should be weighed individually and the maximum safe dose calculated for that individual. Aspiration should always be performed prior to injection to ensure that IV injection is avoided.

Local anesthetics are available in a variety of concentrations with or without adrenaline. Adrenaline causes vasoconstriction and prolongs the action of the local anesthetic. Adrenaline should not be used in animals that have suspect cardiac compromise.

1. **Neonatal Rodent Anesthesia**

A rodent neonate is defined as a mouse <10 days of age. There are several anesthetic methods currently presented in the literature for use in neonatal rodents. These include injectable, inhalant, and physical methods. Hypothermia is the primary physical method utilized in neonatal rodent anesthesia and it is believed to provide anesthesia/analgesia by decreasing neural conduction and synaptic transmission. However, the cooling process itself may be painful and for this reason direct contact with the cooling agent should be avoided. Neonatal rodents demonstrate an increased sensitivity to most injectable anesthetic agents and these have been associated with a high anesthetic mortality in neonates. Inhalant anesthetics are considered safe in neonatal rodents but may have a longer induction time than adult rodents because of their tolerance to hypoxia.

Parental cannibalism is a common problem with neonatal rodent anesthesia. This problem can be reduced by ensuring that the neonate is fully recovered before returning to the dam. Additional steps can also be implemented to reduce cannibalism including smearing pups with soiled bedding from the mother’s cage, placing the pup back in the middle of the litter, and masking scent cues.

1. Anesthesia methods
2. Physical Methods- Hypothermia- can only be performed in Neonatal rodents < 6 days old and should not be used for procedures lasting longer than 30 minutes.
3. Place neonates either on a latex covered bed of crushed ice, in a cut off finger of a latex glove and place in ice water (animal’s head must be held above water to prevent water aspiration and death) or a paper lined test tube and placing in crushed ice/ice water.
4. Animals have reached proper plane of anesthesia when pedal reflex is lost (animal does not respond to toe pinch).
5. Once proper plane is reached animals are removed from ice bath and placed on a chilled cold pack or bed of ice.
6. Use fiber optic light during procedure because incandescent bulbs can warm surgical field.
7. Following anesthesia animal should be re-warmed slowly. Rapid warming can cause tissue damage. Patient can be re-warmed on a circulating water heating pad (40oC) or in an incubator (33oC).
8. Pups can be returned to dam once they are able to crawl.
9. Inhalant anesthetics

Table 1. Inhalant Anesthetics

|  |  |  |  |
| --- | --- | --- | --- |
| Stage of Anesthesia | Route | Oxygen (L/min.) | Isoflurane (%) |
| Induction | Mask or Chamber | 0.5-1 | 4-5 |
| Maintenance | Mask | 0.5-1 | 1-2 |

1. Injectable Anesthetics

Ketamine/Xylazine- Mice >7 days, 50-150 mg/kg Ket + 5-10 mg/kg Xylazine

Intraperitoneal (IP) 27 g needle, 1 ml syringe; maximum volume 0.5 ml

Subcutaneous (SC) 27 g needle, 1 ml syringe; maximum volume 1 ml

1. Emergency Resuscitation

Attempts at resuscitating mice that have received an excessive dose of anesthetic or are experiencing cardiac or respiratory arrest for any reason, are typically unrewarding. Chest compressions often do not restore circulation, and artificial ventilation is difficult in the mouse. A rubber bulb with attached tubing large enough to fit over the nose may be used to periodically inflate the lungs. Respiratory depression can be treated by the administration of doxapram (Dopram®) 5-10 mg/kg IV or IP. If respiratory depression reoccurs, the doxapram should be administered repeatedly at approximately 10-15 minute intervals. Supportive care for animals which reach too deep a level of anesthesia includes raising the body temperature to normal, providing supplemental oxygen through a facemask or nosecone, and administering reversal agents if available (e.g. Yohimbine at 2.1 mg/kg IP or atipamazole 1-2.5 mg/kg IP or SC as needed to reverse xylazine or medetomidine)

**Additional Contacts**

|  |  |  |  |
| --- | --- | --- | --- |
| ***Subject*** | *Contact* | *Phone* | *Email* |
| Veterinary Concerns | LAR Veterinarians | 855-2356 | lar@indiana.edu |
| Policy | IACUC Administrator | 855-5138 | biacuc@indiana.edu |
|  |  |  |  |
|  |  |  |  |

**References**

ACLAM July 2006 Guidelines for the Assessment and Management of Pain in Rodents and Rabbits.

Arras M, Autenried P, Rettick A, Spaeni D, Rulicke T. 2001. Optimization of intraperitoneal injection anesthesia in mice: drugs, dosages, adverse effects, and anesthesia depth. Comp Med 51: 443-456.

Bonaparte (Convenor) D, Cinelli P, Douni E, Herault Y, Maas A, Pakarinen P, Poutanen M, Lafuente MS, Scavizzi F. FELASA guidelines for the refinement of methods for genotyping genetically-modified rodents: A report of the Federation of European Laboratory Animal Science Associations Working Group. *Lab Anim.* 2013; 47(3): 134-145.

Braden GC, Brice AK, Hankenson FC. Adverse Effects of Vapocoolant and Topical Anesthesia for Tail Biopsy of Preweanling Mice. *JAALAS*, May 2015; 54(3): 291-298.

Castelhano-Carlos MJ, et. al. 2010. Identification methods in newborn C57BL/6 mice: a developmental and behavioral evaluation. *Lab Anim.* 1-16.

Dahlborn K, Bugnon P, Nevalanen R, Raspa M, Verbost P, Spangenberg E. Report of the Federation of European Laboratory Animal Science Associations Working Grou on animal identification. *Lab Anim.* 2013; 47: 2-11.

Diesch TJ, Mellor DJ, Johnson CB, Lentle. 2009. Electoencephalographic responses to tail clamping in anesthetized rat pups. Lab Anim 43(3): 224-31.

Garrels W, Cleve N, Niemann H, Kues WA. Rapid non-invasive genotyping of reporter transgenic mammals. *Biotechniques.* May 2012, p 1-4.

*Guide for the Care and Use of Laboratory Animals*, National Research Council, 8th Edition, pp 120-123.

Hankenson FC, et al. 2008. Evaluation of tail biopsy collection in laboratory mice (*Mus musculus*): vertebral ossification, DNA quantity, and acute behavioral responses. *JAALAS*; 47(6): 10-18.

Hankenson FC, Braden-Weiss GC, Blendy JA. Behavioral and Activity Assessment of Laboratory Mice (*Mus musculus*) After Tail Biopsy Under Isoflurane Anesthesia. *JAALAS,* Sept. 2011; 50(5): 686-694.

Jones CP, Carver S, Kendall LV. Evaluation of Common Anesthetic and Analgesic Techniques for Tail Biopsy in Mice. *JAALAS*; Nov. 2012; 51(6): 808-814.

NIH “Guidelines for Toe Clipping of Rodents” [Internet]. May 2010. Available at <http://oacu.od.nih.gov/ARAC/>

NIH “Guidelines of the Genotyping of Mice and Rats”. May 2010; <http://oacu.od.nih.gov/ARAC/documents/Rodent_Genotyping.pdf>

Norecopa “Supplementary Statement on Toe Clipping in Rodents”. March 19, 2010. <http://www.norecopa.no/norecopoa/vedlegg/Supplementary-statement-190310.pdf>.

Paluch LR, Lieggie CC, Dumont M, Monette S, Riedel ER, Lipman NS. Developmental and Behavioral Effects of Toe clipping on Neonatal and Preweanling Mice with and without Vapocoolant Anesthesia. *JAALAS*. 2014 Mar; 53(2): 132-140.

Philbert RA, Zadorozhnyaya I, Beach SRH, Brody GH. A Comparison of the Genotyping Results Using DNA Obtained from Blood and Saliva. *Psychiatr Genet*. 2008 December; 18(6): 275-281.

Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002. Health Research Extension Act of 1985, Public Law 99-158, Animals in Research, Principle V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

Schaefer DC, et al. 2010. Analysis of physiological and behavioral parameters in mice after toe clipping as newborns. *Lab Anim.* 44:7-13.

Symonds EL, Fenech M. A method for non-invasive genotyping of APCmin/+ mice using fecal samples. *Biol Proced Online.* 2012. 14: 1, Jan. 30, 2012.

University of Tennessee Mouse and Rat Toe Clipping. <https://www.uthsc.edu/research/compliance/iacuc/documents/mouse-and-rat-toe-clipping.pdf>

University of Pennsylvania IACUC Guideline Rodent Identification. <http://www.upenn.edu/regulatoryaffairs/Documents/iacuc/guidelines/iacucguideline-rodentidentification.pdf>

**Buprenorphine Dilution and Dosage Chart**

**Buprenorphine (Buprenex®)** 0.3 mg/ml in boxes of 5 1 ml vials

**Dilution for Mice**: 1.0 ml Buprenorphine (0.3 mg buprenorphine/ml) + 9.0 D5W (5% dextrose in water) for injection to make a final concentration of 0.03 mg/ml. Using this dilution, dose mice according to the following chart. Buprenorphine is **light sensitive** so prepare dilution in an **amber bottle** or cover bottle with **foil**.

|  |  |  |  |
| --- | --- | --- | --- |
| **Mouse** | **Dosage** | | |
| **Weight** | **0.05 mg/kg** | **0.075 mg/kg** | **0.1 mg/kg** |
| 15 g | 0.025 ml | 0.04 ml | 0.05 ml |
| 20 g | 0.03 ml | 0.05 ml | 0.07 ml |
| 25 g | 0.04 ml | 0.06 ml | 0.08 ml |
| 30 g | 0.05 ml | 0.08 ml | 0.1 ml |
| 35 g | 0.06 ml | 0.09 ml | 0.12 ml |
| 40 g | 0.07 ml | 0.1 ml | 0.13 ml |
| 45 g | 0.08 ml | 0.11 ml | 0.15 ml |
| 50 g | 0.08 ml | 0.12 ml | 0.17 ml |

Stable for up to 30 d at 21oC or 4oC- Jappinen A, Kokki H, Naaranlahti TJ, Rasi AS. Stability of buprenorphine, haloperidol and glycopyrrolate mixture in 0.9% sodium chloride solution. Pharm World Sci. 1999: 21(6): 272-4.

**Dilution for Carprofen**

**Carprofen (Rimadyl®)** 50 mg/ml 10 ml bottle

**Diluent:** 5% Dextrose (D5W)

**Stability:** stable up to 7 days stored at 4oC, protected from light (amber vials).

**Dilution for Mice:** 1.0 ml carprofen (50 mg/ml) + 49.0 ml D5W (5% dextrose) to make a final concentration of 1 mg/ml. Using this dilution, dose mice according to the following chart.

|  |  |
| --- | --- |
| **Mouse** | **Dosage** |
| Weight | 5 mg/kg |
| 15 g | 0.08 ml |
| 20 g | 0.1 ml |
| 25 g | 0.12 ml |
| 30 g | 0.15 ml |
| 35 g | 0.18 ml |
| 40 g | 0.2 ml |
| 45 g | 0.22 ml |
| 50 g | 0.25 ml |

Solutions stable for **1 week** refrigerated at 4oC.

**Ketamine/Xylazine Dilution for Rodents**

**Ketamine (Ketaset®)** 100 mg/ml in 10 ml vial

**Xylazine (Rompun®, Anased®)** 20 mg/ml or 100 mg/ml 20 ml vial

**Diluent:** 5% Dextrose (D5W) or normal saline (0.9% NaCl)

**Stability:** stable for 28 days stored under ambient conditions and at 4oC, protected from light (amber bottle).

**Mouse Anesthetic Dose**

Ketamine (100 mg/kg) + Xylazine (10 mg/kg)

* 1. **ml Ketamine (100 mg/ml) + 0.5 ml xylazine (20 mg/ml) + 8.5 ml D5W or normal saline for injection OR + 0.1 ml xylazine (100 mg/ml) + 8.9 ml for injection**

**Mice receive 0.1 ml/10 g body weight**

Ketamine and xylazine diluted as above with D5W (5% dextrose) or normal saline are chemically and physically stable after storage for 28 days under ambient conditions of 4oC protected from light.

**Triple Sedative (Ketamine + Xylazine + Acepromazine)**

4 ml Ketamine 100 mg/ml + 1 ml Xylazine (20 mg/ml) + 1 ml Acepromazine (10 mg/ml)=

66.66 mg/ml Ketamine + 3.33 mg/ml Xylazine + 1.66 mg/ml Acepromazine

**0.01-0.03 ml/100g mouse**

**Atipamazole (Antisedan®) Dilution and Dosage Chart**

To Reverse Medetomidine (Dormitor®) or Xylazine (Rompun®)

**Atipamazole (Antisedan®)** 5 mg/ml 10 ml vial

**Diluent:** normal saline (0.9% NaCl)

**Stability:** stable for 28 days under ambient conditions and at 4oC, protected from light (amber bottles).

**Dilution for Mice**

**0.2 ml atipamezole (5 mg/ml) + 4.8 ml sterile saline** to make final concentration of **0.2 mg/ml** solution. This makes a 5 ml dilution of atipamezole which is enough to reverse medetomidine in approximately 25-35 mice weighing between 25-40 g.Using this dilution,dose mice at **0.05 ml solution/10 g body weight SC** according to the following chart.Dose is administered as **1 mg/kg** atipamezole SC.

|  |  |
| --- | --- |
| **Mouse** | **Dosage** |
| **Weight** | **1 mg/kg** |
| 10 g | 0.05 ml |
| 15 g | 0.08 ml |
| 20 g | 0.10 ml |
| 25 g | 0.13 ml |
| 30 g | 0.15 ml |
| 35 g | 0.18 ml |
| 40 g | 0.2 ml |
| 45g | 0.22 ml |

**Bupivicaine Dilution for Rodents**

**Bupivicaine (Sensorcaine®, Marcaine®)** 0.5% (50 mg/ml) 20 ml bottle?= $3.32

**Dilution for Mice**

* 1. ml bupivacaine (50 mg/ml) + 19.9 ml 0.9% saline to make a final concentration of 0.25 mg/ml solution. Using this dilution, dose mice at 0.1 ml solution/mouse (25 g). Dose is administered as **1-2 mg/kg** bupivacaine.

|  |  |  |
| --- | --- | --- |
| **Mouse** | **Dosage** | |
| **Weight** | **1 mg/kg** | **2 mg/kg** |
| 10 g | 0.04 ml | 0.08 ml |
| 15 g | 0.06 ml | 0.12 ml |
| 20 g | 0.08 ml | 0.16 ml |
| 25 g | 0.1 ml | 0.2 ml |
| 30 g | 0.12 ml | 0.24 ml |
| 35 g | 0.14 ml | 0.28 ml |
| 40 g | 0.16 ml | 0.32 ml |
| 45 g | 0.18 ml | 0.36 ml |