

## Rodent Anesthesia Recommendations

Anesthesia is commonly used in mice and rats for a variety of procedures ranging from the collection of some blood samples to performing major surgery. Anesthesia causes a dose-dependent depression of physiologic homeostasis and the degree of anesthesia is dependent on many factors including the species/strain, individual animal variations, procedure being performed, etc.

It is also important to realize that anesthesia is not synonymous with analgesia. Animals may lose consciousness and still be able to perceive pain. For many agents, the plane of anesthesia is therefore paramount. Animals in a light plane of anesthesia may be unconscious but able to perceive pain and stimuli might arouse them. Animals in a deeper, surgical plane of anesthesia, will be both unconscious and lose their ability to feel pain.

Some anesthetic may also have analgesic properties that last beyond the period of anesthesia. However, some agents do not and therefore provision of perioperative and postoperative analgesics become important to best control and animal's discomfort following potentially painful procedures.

The choice of anesthetic is dependent on many factors including: interactions with experimental protocol, duration and level of anesthesia required, required analgesia, etc. The following serve as recommendations for various drugs and should be used as a guide when determining the best anesthetic regiment for specific procedures.

### Inhalant Anesthetics:

Drug	Dose	Route	Duration of Effect	Notes
Isoflurane	1-5% inhaled to effect	Inhaled using a precision vaporizer	As long as animal remains on inhalant	- Survival surgery requires concurrent analgesia - Use precision vaporizer

<sup>1</sup>Controlled substance storage and use must be consistent with NIH Manual 1345: Handling and Safeguarding of Controlled Substances for Nonhuman Use

Mouse Injectable Anesthetics (for dilutions, see below):

Drug/Combo	Dose & Route	Duration of Effect	Notes
Ketamine & Xylazine	Ketamine: 80-100 mg/kg  Xylazine: 5-10 mg/kg  IP	20-30 minutes	- Provides surgical plane of anesthesia with some analgesia - May not be sufficient for some major procedures - Redose with ½ quantity of ketamine ONLY - Can also boost with isoflurane - Reverse xylazine with yohimbine or atipamezole
Ketamine & Dexmedetomidine	Ketamine: 50-75 mg/kg  Dex: 0.5-1 mg/kg  IP	30+ minutes	- Provides surgical plane of anesthesia with some analgesia - May not be sufficient for some major procedures - Redose with ½ quantity of ketamine ONLY - Can also boost with isoflurane - Reverse dexmedetomidine with atipamezole
Ketamine & Xylazine & Acepromazine	Ketamine: 60-100 mg/kg  Xylazine: 5-15 mg/kg  Acepromazine: 1-3 mg/kg  IP	60+ minutes	- Provides prolonged surgical plane of anesthesia with some analgesia - Redose with ½ quantity of ketamine ONLY - Can also boost with isoflurane - Reverse xylazine with yohimbine or atipamezole
Ketamine & Xylazine & Buprenorphine	Ketamine: 60-100 mg/kg  Xylazine: 5-15 mg/kg  Buprenorphine: 0.5-1.0 mg/kg  IP	30+ minutes	- Enhanced analgesia - May improve depth of anesthesia for some procedures and improved length
Sodium Pentobarbital	40-60 mg/kg	20-40 minutes	- Very expensive - Limited analgesia

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## Notes:

- 1) The combination of ketamine and xylazine is popular for the anesthesia of many species including mice. Ketamine is a DEA scheduled substance<sup>1</sup> that, when Combined with a second drug like the sedative xylazine, can provide surgical levels of anesthesia. The most effective dose varies with strain, sex, and age of the animal.
- 2) Ketamine and xylazine alone may not provide a surgical level of anesthesia. Addition of acepromazine or an analgesic such as buprenorphine may enhance the properties and increase both the duration and efficacy
- 3) Ketamine may also be combined with other agents such as dexmedetomidine. This provides some benefits including enhanced reversal and potentially longer duration.
- 4) The responsible anesthetist must determine if sufficient levels and duration of anesthesia have been obtained (such as by evaluating a toe pinch), since underdosing may result in unnecessary pain and overdosing may result in death of the animal. The LASP veterinary staff may assist in determination of the optimal injectable ketamine combination for use in experimental animals.
- 5) All of the above cocktails should be diluted. The tables below provide some guidance on diluting the drugs into a single injectable cocktail administered IP. Dilution is important to ensure adequate dosing and distribution. Dilution may affect a drug's stability, and diluted drugs are typically considered stable for up to one week unless otherwise indicated from applicable reference material. Therefore the mix date should be written on the diluted vial.
- 6) Dilution should be prepared with pharmaceutical grade saline or sterile water. Laboratory-prepared saline or PBS for parenteral injections does not meet this standard. Any use of non-pharmaceutical grade products due to scientific necessity or unavailability of pharmaceutical grade products must be reviewed and approved by the NCI ACUC, as specified in the NIH ARAC Guideline on Pharmaceutical Grade Compounds ([http://oacu.od.nih.gov/ARAC/documents/Pharmaceutical\\_Compounds.pdf](http://oacu.od.nih.gov/ARAC/documents/Pharmaceutical_Compounds.pdf)). Parenteral injections to study animals must be sterile and used prior to the labeled expiration date.
- 7) Avertin is not recommended, but it may be used with a scientific justification approved in the Animal Study Proposal. Use should be in line with the ACUC Policy "Use of Tribromoethanol (Avertin) in Rodents" (<https://ncifrederick.cancer.gov/lasp/acuc/bethesda/Media/Documents/UseOfTribromoethanol.pdf>)

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## Ketamine/Xylazine:

	<b>Drug stock concentration<sup>1</sup> (mg/ml)</b>	<b>Volume used for cocktail</b>	<b>Volume of cocktail administered to mouse per 10 g body weight</b>	<b>Dose administered to mouse</b>
Ketamine	100 mg/ml	1.0 ml	-	<b>100 mg/kg</b>
Xylazine	20 mg/ml	0.5 ml	-	<b>10 mg/kg</b>
Saline	Sterile, isotonic	8.5 ml	-	-
Combination Cocktail		10 ml total	<b>0.10 ml / 10 g</b>	-

## Ketamine/Xylazine/Acepromazine:

	<b>Drug stock concentration<sup>1</sup> (mg/ml)</b>	<b>Volume used for cocktail</b>	<b>Volume of cocktail administered to mouse</b>	<b>Dose administered to mouse</b>
Ketamine	100 mg/ml	0.8 ml	-	<b>80 mg/kg</b>
Xylazine	20 mg/ml	0.3 ml	-	<b>6 mg/kg</b>
Acepromazine	10 mg/ml	0.1 ml	-	<b>1 mg/kg</b>
Saline	Sterile, isotonic	8.8 ml	-	-
Combination Cocktail		10 ml total	<b>0.10 ml / 10 g</b>	-

## Ketamine/Xylazine.Buprenorphine

	<b>Drug stock concentration<sup>1</sup> (mg/ml)</b>	<b>Volume used for cocktail</b>	<b>Volume of cocktail administered to mouse</b>	<b>Dose administered to mouse</b>
Ketamine	100 mg/ml	0.8 ml	-	<b>80 mg/kg</b>
Xylazine	20 mg/ml	0.3 ml	-	<b>6 mg/kg</b>
Buprenorphine	0.3 mg/ml	1.7 ml	-	<b>0.5 mg/kg</b>
Saline	Sterile, isotonic	7.2 ml	-	-
Combination Cocktail		10 ml total	<b>0.10 ml / 10 g</b>	-

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## Ketamine/Dexmedetomidine:

	<b>Drug stock concentration<sup>1</sup> (mg/ml)</b>	<b>Volume used for cocktail</b>	<b>Volume of cocktail administered to mouse</b>	<b>Dose administered to mouse</b>
Ketamine	100 mg/ml	0.3 ml	-	<b>75 mg/kg</b>
Dexmedetomidine	0.5 mg/ml	0.8 ml	-	<b>1.0 mg/kg</b>
Saline	Sterile, isotonic	6.9 ml	-	-
Combination Cocktail		8 ml total	<b>0.2 ml / 10 g</b>	-

**Reversal**

Ketamine is not reversible following injection, but must be metabolized; however both xylazine and Dexmedetomidine are reversible with the injectable product atipamazole (Antisedan, 5 mg/ml). Inject 0.1 to 1.0 mg/kg either subcutaneously (SC) or IP for reversal of the sedative and analgesic effects.

Because of prolonged recovery from anesthesia and marked bradycardia, and Dexmedetomidine reversal is strongly recommended.

**Rat Anesthesia**

Suggested starting dose for rat anesthesia is 80 mg/kg ketamine and 10 mg/kg xylazine.

**Pre-operative Considerations**

Fasting rodents is not necessary and is not recommended. Ophthalmic ointment should be applied to the eyes to prevent drying of the corneas, as ketamine anesthesia suppresses the blink reflex.

**Post-operative Considerations**

- The animals should be kept dry, insulated, or warmed to prevent excessive loss of body heat. Hypothermia is typical during and following surgery and can contribute to mortality.
- Surgical records for research rodents must be maintained and should be individual or cage specific. The record should reflect the procedure performed and date, dates and doses of anesthetics and analgesics delivered, and the initials of the person administering them. LASP recommends keeping this data on the back of the cage card and has templates available.

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