

Lab Animal, Mouse and Rat (Gas) Anesthesia Protocol:

A) Gas Anesthesia

Gas anesthesia is the preferred method of immobilization for in vivo imaging of mice and rats.

*Isoflurane gas anesthesia is recommended due to the fact that isoflurane is minimally metabolized (<0.17%) by the liver and therefore is less toxic to the animal's metabolism as compared to injectable anesthetics.

Materials:

- Rodent gas anesthesia instrument equipped with a vaporizer, charcoal scavenger filters, induction chambers and nose cones.
- Isoflurane anesthesia gas.

Methods:

- Weigh scavenger filter and record (saturation weight is indicated).
- Dial vaporizer to "0"/OFF and fill with isoflurane.
- Turn on O2 tank at 55psi.
- Set O2 flow meter to 1l/min.
- Place animals in induction chamber.
- Induce anesthesia – dial vaporizer to 3%; animals will sedate in 1-2 min.
- Reduce anesthesia to 1.5-2% (imaging is non-traumatic only a low dose of anesthetic is required to immobilize the animal; consistency of anesthesia dosing procedures is recommended in order to reduce variability in anesthetic effects).
- Cover eyes with eye lubricant in order to prevent corneal dehydration (isoflurane inhibits blinking reflex).
- Transfer animals to imaging chamber and place in front of individual nose cones.
- Maintain animals warm during anesthesia in order to prevent hypothermia.

Recovery:

- Turn vaporizer dial to "0"/OFF.
- Keep O2 on for a couple of minutes to facilitate recovery.
- Animals recover within 1 min.
- Remove animals.
- Turn off O2 tank and flush anesthesia system to remove caustic gas from system.
- Clean and sanitize equipment.

B) Injectable Anesthesia:

*Do not use more than once a day. Liver toxicity is otherwise lethal.

1) Ketamine/Xylazine is the most commonly used injectable anesthetic cocktail.

- **Mouse:** Dose at 50-100 mg/kg of Ketamine-HCl and 5-10mg/kg Xylazine-HCl, intraperitoneal (IP) injection. Duration of effect ~30 min.

- **Rat:** Dose at 60 mg/kg of Ketamine-HCl and 5-10mg/kg Xylazine-HCl, IP injection. Duration of effect ~30 min.

Practical Protocols:

- Mouse anesthesia: Ketamine-HCl (100 mg/ml), and Xylazine-HCl (20mg/ml), mixed at a 4:1 (vol/vol) ratio just before use. (0.03ml/20g mouse IP). If necessary, a second dose of 7 ul per 10g of body weight can be given, but this increases the risk of over dose. OR,
- Mouse anesthesia: In a sterile 10 ml bottle with a rubber stopper, mix 1 ml of ketamine (100 mg/ml), 0.1 mL of xylazine (100 mg/ml), and 8.9 mL of sterile water for injection. Shake well before use. Keep away from light and in a cool place. Inject 0.1 ml/10 g IP Repeat half a dose at a time whenever necessary (approximately every 30 min). Prevent heat loss until the animal recovers.
- Rat anesthesia: Similar to the mouse anesthesia method, but mix 8.75 ml of ketamine (100 mg/ml) and 1.25 ml of xylazine (100 mg/ml). Administer 0.05–0.10 ml/100 g IP Repeat as required with 1/3 to 1/2 doses at a time (approximately every 30 min).

2) Avertin

- Mouse: Dose at 200-240mg/kg; IP injection; duration of effect 15-30 min.

Avertin stock is a mixture of 25g avertin (2-2-2 tribromoethanol, Aldrich) dissolved in 15.5 ml tert-amyl alcohol (2-methyl-2-butanol; Fisher Scientific), store at room temperature in the dark. For a working solution, 0.5 ml avertin stock is mixed with 39.5ml 0.9% saline or PBS to 20 mg/ml. Sterilize with Nalgene 0.22 µm filter bottle and use fresh – light sensitive.

3) Pentobarbital Sodium

- Mouse and Rat: 50 mg/kg; IP injection; duration of effect 20-60 min; no analgesia.

4) Ketamine/Medetomidine

- Mouse and Rat: 75 mg/kg and 1 mg/kg; IP injection; duration of effect 30-40 min.

- Anesthesia can be reversed with 1 mg/kg atipamezole.

6) Chloral hydrate

- Mouse: 400 mg/kg; IP injection.

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