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Rat Isoflurane Anesthesia Protocol

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This protocol is implemented and regularly used.

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Abstract

This protocol provides a detailed guide to set up anesthesia using an Isoflurane vaporizer system before performing surgical procedures in rats.

Safety warnings

- ⚠ This protocol involves the use of isoflurane and oxygen for anesthesia. Isoflurane is an anesthetic that can cause respiratory depression or dizziness if inhaled, and oxygen supports combustion. All procedures should be conducted in a well-ventilated area or under an active scavenging system to prevent personnel exposure to anesthetic gases. Ensure all connections are secure, and oxygen cylinders are properly regulated, chained to the wall, and kept away from heat or open flames. Wear appropriate personal protective equipment (PPE).

Ethics statement

All procedures involved were conducted per the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, San Diego.

Preparing Oxygen

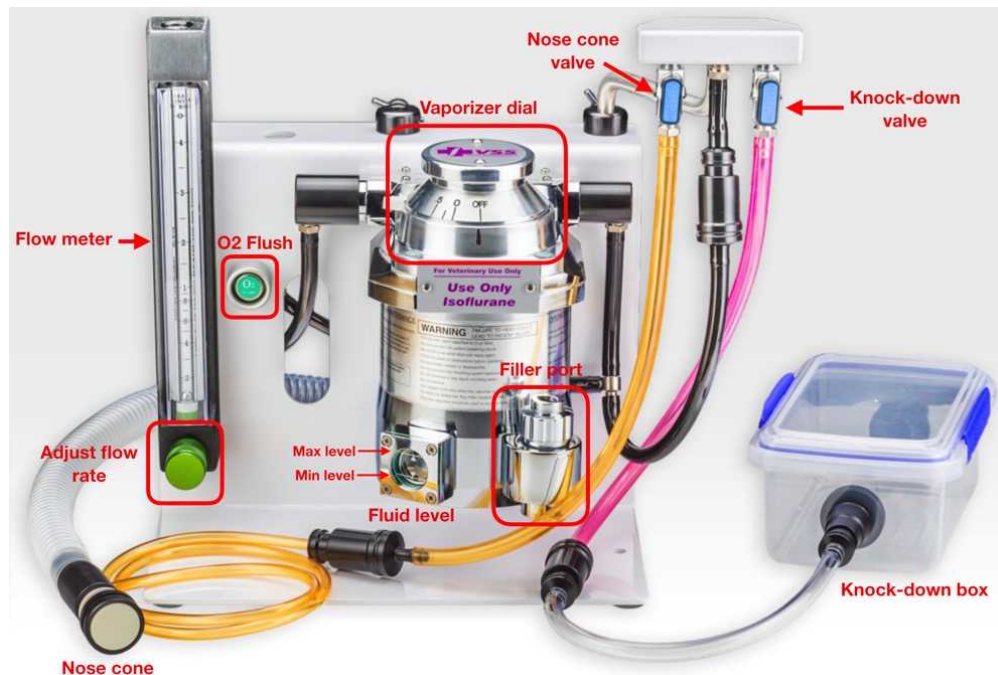
- 1 Turn on main oxygen line in the back right corner of the room. An audible rush of air should be heard upon opening the canister.
- 2 At the surgical workstation, turn on the green knob of the oxygen regulator until the steel bead hovers at 2.5, as visualized in the attached image. Audible air moving sounds confirms that oxygen is flowing.



Steel bead of Oxygen Regulator is at 2.5

Preparing Isoflurane vaporization

- 3 Ensure all tubing and equipment is set up as shown in the diagram. This protocol will describe how to prepare and refill the **Drager Vapor 2000 Isoflurane model** and the **VIP 3000 Veterinary Vaporizer model**.



Schematic of Anesthesia setup.

- 4 Ensure that the isoflurane vaporizer's isoflurane level is between the minimum and maximum fill lines.



Minimum and Maximum fill lines of Dräger Vapor 2000 Isoflurane model.



Minimum and Maximum fill lines of VIP 3000 Veterinary Vaporizer model.

5 Refilling a Drager Vapor 2000 Isoflurane model:



5.1 Open bracket-slide and pull purple key handle out and down.



Open bracket-slide.



Pull out purple key.

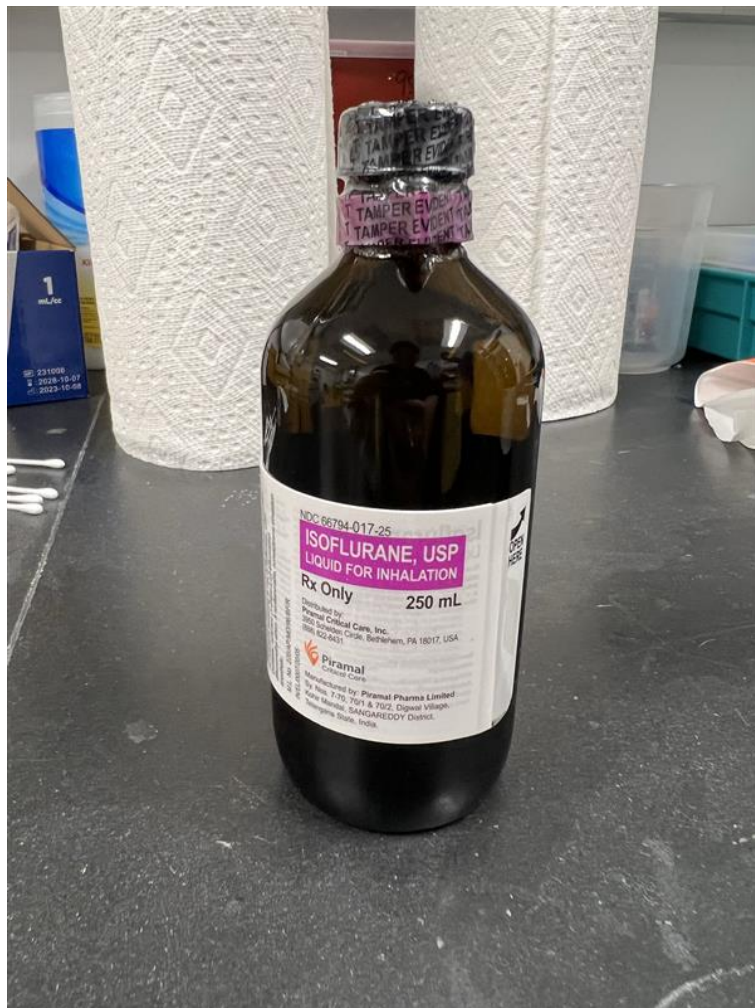


Purple key should be fully out.

5.2 Attach purple tube attachment to isoflurane bottle.



Note: Isoflurane may cause skin and eye irritation, and proper precaution should be used when handling or refilling vaporizing devices. Avoid breathing vapors, as they may cause dizziness and drowsiness.



New Isoflurane bottle.



Purple filling tube attachment screwed onto top of the isoflurane bottle.

- 5.3 Connect the purple tube attachment end to the vaporizer opening, making sure to push all the way in.



Filling tube inserted into keyhole slot on vaporizer.

5.4 Invert isoflurane bottle to fill vaporizer.



Inverted isoflurane bottle to fill vaporizer.

5.5 When finished, remove tube connector and isoflurane bottle, push purple key handle back in, and close bracket-slide.



Re-inserting filling key into vaporizer after filling.

- 6 To turn on anesthesia flow in **Dräger Vapor 2000 Isoflurane** model, push on Zero button while turning dial counter clockwise.



Push down Zero button and twist dial counterclockwise to open anesthesia flow.

- 7 Refilling a **VIP 3000 Veterinary Vaporizer** model:

- 7.1 Unscrew isoflurane well lid in counter-clockwise direction.



Opening well to refill isoflurane.

- 7.2 Pour isoflurane directly into well opening, checking sight glass to ensure isoflurane is between the minimum and maximum mark lines.



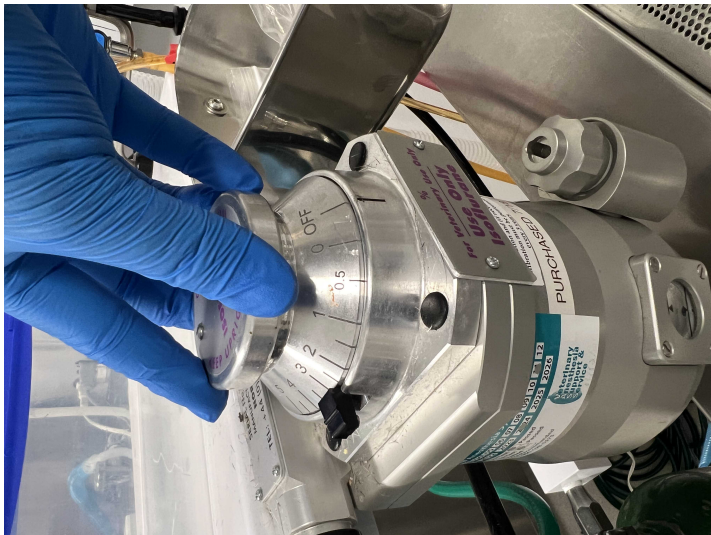
Pour isoflurane directly from bottle into well.

- 7.3 When finished, tighten well lid in clockwise direction.

- 8 To turn on anesthesia flow in **VIP 3000 Veterinary Vaporizer** model, push down side lever while turning dial counter clockwise.



Push down side lever.



Turn dial counterclockwise to open anesthesia flow.

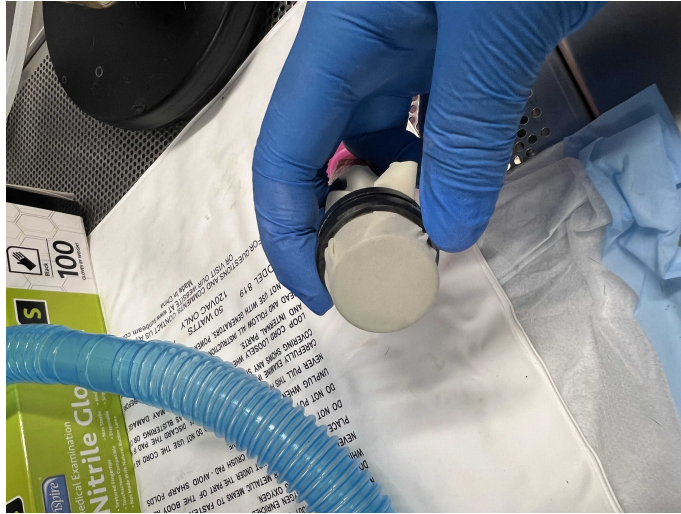
- 9 Using a balanced scale, weigh the **F/Air Canister** to ensure the gained weight is less than 50 grams. If not, replace the canister, ensuring the new one is weighed and weight recorded before first use.

Connect the F/air to the nose cone valve (orange tubing). Ensure the canister is on a flat, level surface during the entirety of the procedure.



Proper configuration of the isoflurane vaporizer with the F/air canister.

- 10 Attach a new nose cone elastic vapor shield before starting a new procedure, making sure a slit is adequately made to fit the animal.



Appearance of new elastic vapor shield (without slit) on the nose cone.



Appearance of new elastic vapor shield (with slit) on the nose cone.

Inducing and Maintaining Anesthesia

- 11 Before inducing anesthesia to any animals, ensure the proper documentation for the procedure is complete according to IACUC or protocol guidelines. This may include the exact length of the procedure, as well as the notation of any drugs administered to the animal beforehand (e.g. Cefazolin, Flunixin, ophthalmic ointment, etc).

In this case, the George Lab surgical record log and ACP surgical log both need to be completed before and after the procedure.



- 12 To induce anesthesia, turn on the Isoflurane vaporizer, and turn to the "5%" mark. This level will induce anesthesia for both male and female animals inside of the knock-down chamber. Allow animal to be anesthetized at this level with close observation for 5 minutes. **Always monitor breathing rate of the rats, and do not keep them in the knock-down box for too long to prevent anesthesia overdose.**

Once the animal is anesthetized, it may be moved to the surgical table.

- 13 To maintain anesthesia throughout the procedure, set the anesthesia flow to between 2-3.5% based on the animal's weight. To ensure proper anesthetic conditions, the target respiratory rate of the animal should be between 70-100 breaths/min, and should be placed on a heating pad underneath a surgical pad to allow body temperature to remain between 35-37°C.
- 14 Before starting any surgical procedure, perform one of the following reflex tests to ensure animal is not conscious. If no reflexes are present, the procedure may begin.
1. Toe pinch
 2. Blinking reflex
- 14.1 **Toe Pinch:** Using a small clamp, pinch the back foot of the animal to observe if a pedal foot withdrawal reflex occurs. In the absence of the reflex, proceed, if the animal withdraws the back foot, allow the animal to remain in the isoflurane chamber at 5% for 2 additional minutes, or until no reflex is observed.
- 14.2 **Blinking reflex:** Using a finger, gently touch the cornea of the animal, and observe if blinking occurs. In the absence of the reflex, proceed, if the animal blinks, allow the animal to remain in the isoflurane knock-down chamber at 5% for 2 additional minutes, or until no reflex is observed.
- 15 Throughout the entirety of the procedure, the animal must be continuously monitored for signs of shallow, irregular, or labored breathing. This indicates the level of isoflurane may be excessive, and needs to be decreased adequately.

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