



RESTORE

*Resources for Expanding Stem
cell-derived Tissues and Organs
for Regenerative Engineering*

STANDARD OPERATING PROCEDURE

RAS-4 Anesthesia

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1. Policy

- 1.1. Any person working off this protocol has previously received training on this specific piece of equipment by the RESTORE Lab Manager or Technical Director with explicit instruction for independent equipment usage. Any persons operating equipment without prior training and/or permission will be removed from the facility immediately.
- 1.2. No food or drink within any of the RESTORE facility.

2. Purpose

- 2.1. The purpose of this procedure establishes standardized requirements for the safe and proper operation of RAS-4 Rodent Anesthesia machine to ensure data quality, user safety, instrument integrity, and regulatory compliance.

3. Responsibilities

- 3.1. The equipment operator is responsible for the proper handling, care, and oversight of all samples, animals, and associated materials while they are under the operator's control during use of the instrument.

4. Procedure

4.1. Isoflurane Check/Refill

- First, verify that the anesthetic fill window is empty/low before refilling (**Fig 1.1A**). If the level is adequate, proceed to the next section. If refilling is required: **VAPORIZER IS UNDER PRESSURE! BLEED PRESSURE BEFORE FILLING!**
 - Turn off the oxygen supply at the source (green tank located to the left of the IVIS).
 - Set the vaporizer dial to 1%.
 - Turn the O₂ Master Switch off (down position; **Fig 1.2, red box**).
 - Set Circuit #1 and Circuit #2 flow rates to 1000 cc/min (**Fig 1.2, blue arrows**).
 - Wait until the pressure gauge reads 0 psi (**Fig 1.2, green box**).
- Gather the isoflurane bottle and compatible keyed filling adapter (**Fig 1.1B**).
- Attach the adapter to the isoflurane bottle by fitting the keyways of the bottle adapter to the keys of the bottle collar. (**Fig 1.1C**).
- Open the vaporizer filler assembly by unscrewing the top screw (**Fig 1.1D**) and remove the vaporizer fill-port cap (**Fig 1.1E**) exposing the fill port (**Fig 1.1F**).
- Insert the keyed adapter nozzle fully into the vaporizer fill port, the two holes on the key (**Fig 1.1G**) facing downward, and tighten the lock knob clockwise until secure and leak-free.
- Tilt or invert the bottle to start the fluid transfer, watching in the sight glass for the anesthetic level to reach the indicated fill line/appropriate level. (**Fig 1.1H, K**)
- Return the bottle to the upright position, loosen the lock, pull the adapter out, and tightly seal the vaporizer fill port. (**Fig 1.1 I & J**)
- Verify that the anesthetic level is at the FULL mark in the sight glass before operating the system (**Fig 1.1 K**)

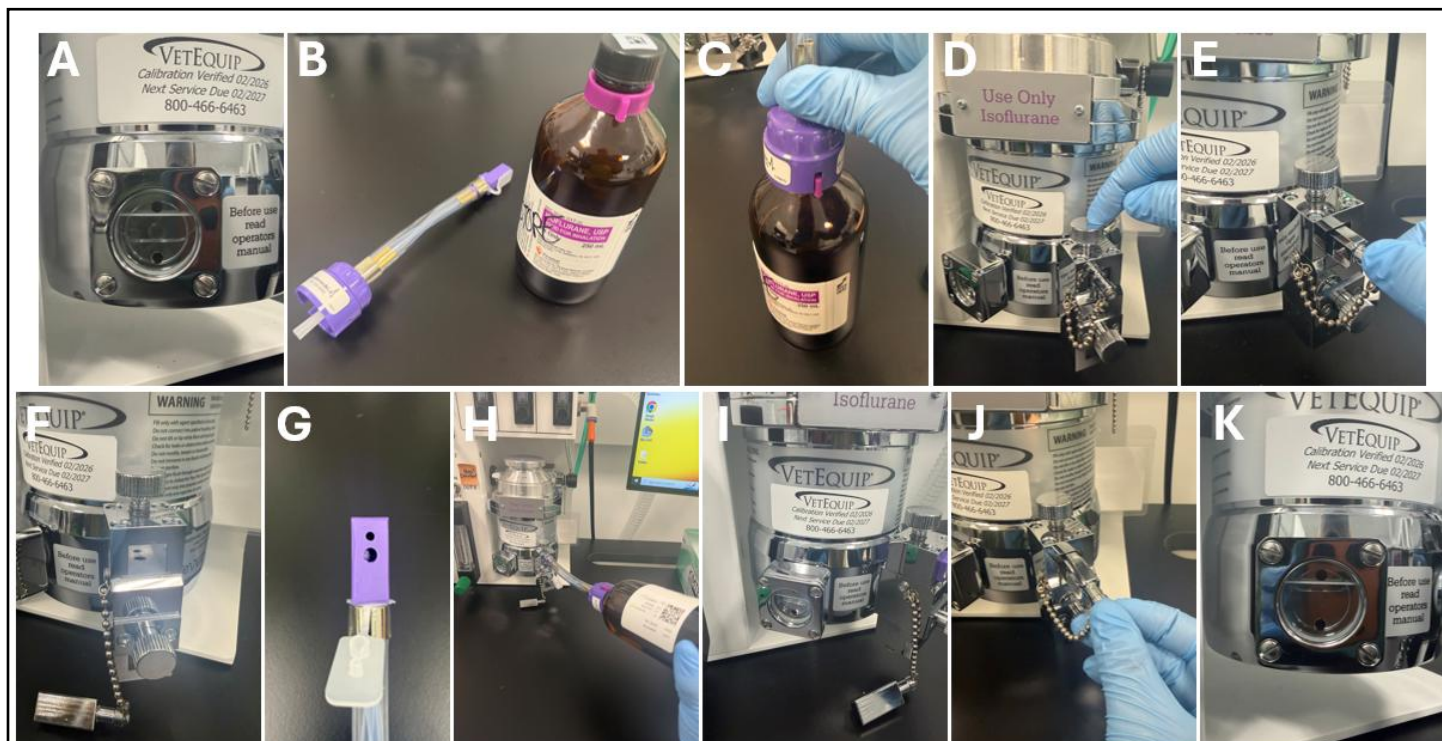


Figure 1.1: Isoflurane vaporizer filling procedure showing the anesthetic level check (A), isoflurane bottle and keyed adapter (B), adapter attachment (C), opening the filler assembly and removing the fill-port cap (D-F), align the adapter in the fill port (G), filling the vaporizer to the indicated level (H), removing the adapter and resealing the fill port (I-J), and verification of a full anesthetic level (K).

4.2. Anesthesia Startup

- Turn ON the oxygen supply at the source (green tank located to the left of the IVIS system).
- Turn the O₂ Master Switch to the ON position. (up; **Fig 1.2, red box**) Verify that the system pressure is 6.0 ± 0.5 PSI. (**Fig 1.2, green box**)
- Enable the desired Anesthesia Delivery Port(s). (**Fig 1.2, blue arrows**)
- Turn ON the Vacuum Pump Master Switch. Confirm that the green light indicator is illuminated. (**Fig 1.2, yellow box**)
- Enable the desired Vacuum Exhaust Control Port(s). (**Fig 1.2, purple arrows**)
 - Note: The anesthesia box vacuum exhaust is continuously active and does not require a switch.
- Set the vaporizer to the desired isoflurane concentration. (**Fig 1.2, pink box**)

4.3. Anesthesia Shutdown

- Turn the vaporizer dial to 0% isoflurane. (**Fig 1.2, pink box**)
- Disable all Anesthesia Delivery Port(s). (**Fig 1.2, blue arrows**)
- Disable all Vacuum Exhaust Control Port(s) that were in use. (**Fig 1.2, purple arrows**)
- Turn OFF the Vacuum Pump Master Switch and verify that the green indicator light turns off. (**Fig 1.2, yellow box**)
- Turn the O₂ Master Switch to the OFF position. (**down; Fig 1.2, red box**)
- Turn OFF the oxygen supply at the source (green tank located to the left of the IVIS system).
- Make sure to wipe the anesthesia box down with disinfectants.
 - Note: Ethanol dries out and causes cracks in plexiglass. DO NOT use Ethanol
- Weigh and record the weight of the isoflurane scavenger.

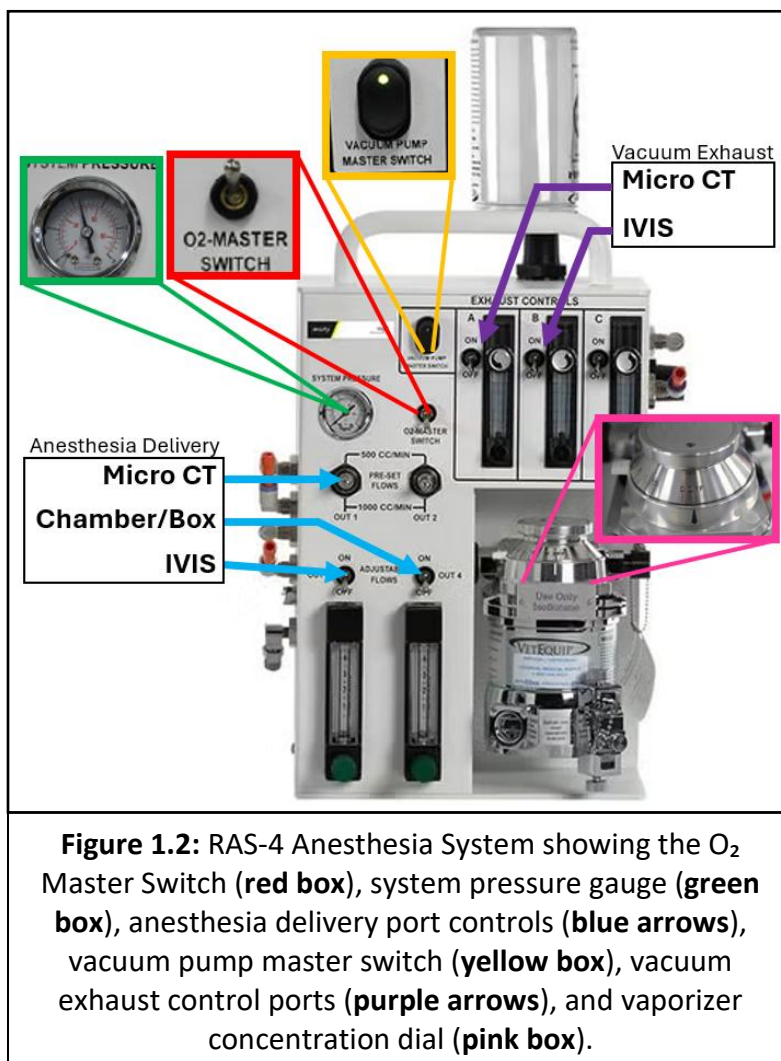


Figure 1.2: RAS-4 Anesthesia System showing the O₂ Master Switch (**red box**), system pressure gauge (**green box**), anesthesia delivery port controls (**blue arrows**), vacuum pump master switch (**yellow box**), vacuum exhaust control ports (**purple arrows**), and vaporizer concentration dial (**pink box**).