

Standard operasjonsprosedyre: Anaesthesia with Isoflurane

SOP nr: 15-01

Opprinnelig dato: 08.01.2021

Revidert dato: 02.08.2023

Gyldig til dato: 02.08.2025

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ANAESTHESIA WITH ISOFLURANE

1.0 PURPOSE

- 1.1 To ensure that the health and safety of all personnel is maintained.
- 1.2 To ensure that the animal is properly handled and anaesthetized with a minimum of stress and effect on its long-term health.
- 1.3 To ensure the proper handling and use of equipment, storage of Isoflurane and disposal of waste.

2.0 PHARMACOLOGY

- 2.1 1-chloro-2,2,2-trifluoroethyl difluoromethyl ether, commonly known as Isoflurane, is a halogenated inhalation general anaesthetic, which is usually used as an animal anaesthetic.
- 2.2 When using Isoflurane for a longer time period you may see symptoms like: Hypotension, respiratory depression and hypothermia. Turn off isoflurane if symptoms occurs and use a heating pad during the procedure.
- 2.3 Isoflurane has no analgesic properties. If a procedure that cause pain is performed, make sure to give analgesics (opioids or NSAID) either before or during the procedure.
- 2.4 Isoflurane is a non-flammable, clear, colourless liquid with a mild ether-like odour. Isoflurane is a very volatile liquid at ordinary temperatures and pressure (its evaporation rate increases with increasing temperature).
- 2.5 It is recommended that the average concentration of halogenated agents should not exceed 2ppm (15mg/m³) during any 1-hour period.
- 2.6 In a fire, toxic substances/products may be released because of the decomposition of Isoflurane (e.g. carbon oxides, hydrogen chloride gas or hydrogen fluoride).

3.0 DIVISION OF RESPONSIBILITY

- 3.1 Everyone planning a pregnancy or who has a suspected/confirmed pregnancy is responsible for informing his or her supervisor of this. KPM is always helpful with advice if needed.
- 3.2 All who handle Isoflurane must be familiar with the Safety Data Sheet (SDS) for the product and use at least the minimum recommended protective equipment.
- 3.3 The head of department at KPM is responsible for approving the procedure and for seeking veterinary advice of relevance for this procedure.
- 3.4 The Head of Department at KPM provides necessary information and training for staff.



- 3.5 The Head of the department at KPM is responsible for ensuring that proper protective equipment is available for KPM staff.
- 3.6 The Head of Department may delegate the tasks described in paragraphs 3.2 – 3-5.
- 3.7 The group leader is responsible for ensuring that users have proper protective equipment and has received proper training in the use.
- 3.8 Manuals are available in the laboratories and on the KPM homepage and must be read and understood before equipment is used. There is also a link to the manuals underneath “8.0 references” in this SOP. KPM provides training for users if needed.
- 3.9 Each user of the facility and KPM employees are responsible for adhering to the procedure at all times.
- 3.10 All malfunctioning equipment must be reported to KPM by e-mail (komparativ@medisin.uio.no).
- 3.11 KPM must carry out regular service on equipment as often as suggested from the supplier.

4.0 PROCEDURE

- 4.1 It is not recommended that pregnant women work with Isoflurane or any other chemicals that might harm the mother and baby. An extensive risk assessment must be completed if this is unavoidable.
- 4.2 The Isoflurane bottle must be marked with the name of the user, the opening date, and stored in a well-ventilated cabinet or in the chemical cabinet in DU-028 (see illustration 1).
- 4.3 When empty, leave the Isoflurane bottle uncorked in the fume hood until any remaining liquid has vaporized, then attach the cork and dispose of the container as hazardous waste.
- 4.4 Use proper gloves that provide protection against chemicals when working with Isoflurane. Additional protection equipment, such as a laboratory coat, safety glasses and mask with a filter for organic vapours is also recommended.
- 4.5 Isoflurane must be administered by an approved and calibrated anaesthetic vaporizer machine, with a filter system, in a well ventilated room equipped with an extractor arm fume hood (see picture 1).
- 4.6 Handle Isoflurane, when possible, in a fume hood, for example when you change from cap to key adapter.
- 4.7 The use of charcoal absorber canisters as passive scavengers must be closely monitored. The manufacturer’s recommendations regarding the canister use time and the proper weight of canisters must be closely followed and should be checked before each use. Place saturated charcoal cartilage in the fume hood. This will be disposed of by KPM.
- 4.8 Use only healthy animals, based on a physical examination and scoring sheet, for non-terminal procedures.
- 4.9 There are individual differences in tolerance due to age, strain etc. Adjust the concentration of Isoflurane based on your observations of the animals’ respiratory pattern and reflexes.
- 4.10 The induction chamber is used to induce anaesthesia. Move animals to the mask when confirmed unconscious.
 - Stage 1 (awake, but affected): disorientation, urination, and defecation.
 - Stage 2 (unconscious): reflexes, rapid, irregular breathing, whiskers moving.
 - Stage 3 (anaesthetized): loss of reflexes, deep rhythmic breathing, whiskers not moving.
 - Stage 4 (overdosed): respiratory arrest, hypotension - resuscitation or drugs to reverse anaesthesia are needed to prevent death.

4.11 Preoperative:

- The animal must be acclimatized to the facility if imported (for up to two weeks in some cases).
- Use only healthy animals for non-terminal procedures.
- Gently shave and disinfect the area for surgery without wetting the fur.
- Administration of an analgesic in any type of invasive surgery where the animal will wake after surgery is mandatory.
- Consider injection of supportive saline solution.
- Use eye gel to prevent eye dryness and damage.
- Inject analgesics if pain is expected.

4.12 Intraoperative:

- Make sure that the animal is deeply anaesthetized before starting surgery by checking its reflexes and respiratory pattern.
- Monitor the animal closely during surgery. Decrease/increase the concentration of Isoflurane according to the condition of the animal by checking reflexes, chest wall movements, heart rate, direct or indirect blood pressure (cuff or Doppler), colour of feet/ears/tongue, temperature and oxygen saturation in blood (using a pulse oximeter).
- Use a heat pad and monitor body temperature. NB! Ensure that the heat pad is not too hot to prevent burns!

4.13 Postoperative:

- Keep animals separated until conscious. Animals should be checked frequently, preferably every 10-15 minutes, turning the animals from side to side until recovered.
- Check hydration status and give supportive saline solution if needed.
- Keep the cage on a heat pad or under a heat lamp until the animal regains consciousness. NB! Make sure you do not overheat the cage!
- Monitor recovery from anaesthesia closely and be prepared to provide respiratory support.
- Monitor food and water intake after recovery from anaesthesia and provide nutritional support if needed. Leave food on the bottom of the cage for easy access and use a gel pack if invasive surgery has been performed.
- Administer analgesic according to instructions and check for signs of discomfort or pain.
- Monitor wounds for infections.
- Use a designated score sheet to score the animal everyday post-op to make sure the animal is healing after surgery.
- Fill out analgesic form when analgesics is given. Place it on the cage, clearly visible for KPM-staff.

4.14 All cages with animals that are to be used in the experiment must be designated as “Experimental” by the user in SL (Science Linker) and relevant information must be added under “Notes”. And a green cage card it so be made for the cage.

4.15 Information about the performed procedure must be added to “Animal examination” in SL.

- 4.16 Completed pre- and post-surgery analgesia record (“Registration of analgesic”) should be available in the room. A score form must be completed and made available in a folder inside the room. Mark the cage card with an “eye sticker” framed with a pink highlighter (available in the room), as a sign to the staff at KPM that additional follow-up is required.
- 4.17 We currently use Harvard Apparatus Anesthetic Vaporizers, for use of the univentor and scavenger contact KPM to receive instructions and manual (manuals can also be found at KPMs homepage).

The use of Harvard Apparatus Anaesthetic Vaporizer (picture 6):

Warnings taken from the supplier [homepage](#):

- Keep the vaporizer upright at all times.
 - Do not carry the vaporizer by holding the dial control.
 - To minimize cross-contamination of anesthetic agents, only one vaporizer should be fitted to an anesthetic machine at any one time.
 - The vaporizer must be always be mounted between the gas flow (fresh gas supply) metering unit and the patient breathing circuit and upstream of any absorber or humidifier.
 - Before use, all connections must be checked for leaks and functional tests must be performed as described in the anesthetic machine User Manual.
 - It is recommended that only one vaporizer is mounted to the anesthetic machine at any one time, thus assisting with the prevention of cross-contamination of anesthetic agents.
 - Do not fill the vaporizer with any liquid of anesthetic agent other than the one specified on the Front Label. The vaporizer is designed for that agent only. Any other agent than that specified can prove dangerous to the patient.
 - Do not fill vaporizers unless the Control Dial is in the "OFF" position.
 - Do not turn the Control Dial "ON" during filling or attempt to fill beyond the "FULL" mark.
 - Do not drain the agent into a container other than a properly marked container.
- 4.18 Follow the “how to use the machine” manual that can be found inside the labs, and on page 12-15 in this SOP. This must be read and understood before using the equipment. For more in-depth about the machine you can also read “[Harvard apparatus Anaesthetic Manual](#)” from the supplier.
- 4.19 The charcoal cartridge must be weighed before start, and should not weigh more than 1400gram. Make sure to turn it on before you use the vaporizer.
- 4.20 To refill the vaporizer use the “key adapter” (picture 7). Fit it to the flask and make sure to close the screw on the vaporizer properly before pouring the isoflurane. Refill the tank with as much as needed for the procedure. Don’t let the isoflurane reach bellow the lower level.
- 4.21 Syringe with a small hose may be used to refill isoflurane, but is not recommended since it may cause air bubbles in the isoflurane and may cause spill.
- 4.22 Make sure the gas flows to either chamber or mask by turning the switch in the correct direction. This may vary from each lab so pay attention to the hoses, notes and manual by the vaporizer.


- 4.23 The machine in DU-051, DU-050 and DU-015 can use both chamber and mask at the same time. Watch the animal on mask closely to avoid the animal receiving too much isoflurane.
- 4.24 Recommended isoflurane dosage (this may vary from strain and individuals):
Isoflurane concentration using induction chamber: Mice and rats – 3-4%
Isoflurane concentration using mask: Mice and rats - 1.5-2.5%
- 4.25 For air settings sett manual on page 12-15 or inside the laboratories.
- 4.26 Wipe off any spills on the anaesthesia machine and filtration unit using paper and a mild detergent. Do not use alcohol or any other solvent.
- 4.27 Wipe the induction chamber and mask with mild soapy water. Ethanol should not be used on the chamber.
- 4.28 Clean the workbench with soapy water and disinfect with 70% ethanol. Sweep the floor. Put away all personal belongings in a cabinet or on shelves.
- 4.29 Wash hands thoroughly with soap and water.


5.0 HEALTH, SAFETY AND ENVIRONMENT (HSE)

- 5.1 Mandatory PPE: two layers of gloves covering sleeves, one layer being chemical resistant. Recommended PPE: safety glasses, a facemask with filter for organic vapours and a laboratory coat.
- 5.2 Exposure to Isoflurane may occur when filling the anaesthetic machine or from the escape of waste anaesthetic gases while administering the anaesthetic. When possible, handle Isoflurane in a fume hood. Use the scavenging system and the extractor arm fume hood.
- 5.3 Rinse eyes thoroughly if Isoflurane gets into the eyes.
- 5.4 Remove clothes and shoes if Isoflurane is spilled. Rinse the skin thoroughly with water.
- 5.5 Evacuate yourself and others from the room if larger spills occur. If proper protective equipment and a face mask with a filter for organic vapours is worn, soak up spills with a sponge or paper and place in the fume hood. Place outer gloves in fume hood. When vaporized, place paper/sponges and gloves in a dual bag, close with a knot and discard as hazardous waste.
- 5.6 Call 113 in an emergency.
- 5.7 All HSE breaches must be reported in Cim.
- 5.8 Acute exposure: Inhalation of high concentrations (3%) may lead to death by medullary paralysis. Those recovering from exposure may suffer from shivering, nausea, vomiting, ileus, or excitation, and there may be a transient white blood count increase. A slight decrease in intellectual function may persist for 2-3 days, with small mood changes or symptoms possible for 6 days. Induction of general anaesthesia may cause malignant hyperthermia from hyper metabolism of skeletal muscles in susceptible individuals.
- 5.9 Long-term exposure could potentially lead to liver/kidney disease, cancer, sterility, miscarriages and birth defects in offspring. Short-term exposure can lead to headaches, nausea, dizziness, fatigue and respiratory irritation.
- 5.10 Inhalation of Isoflurane at a concentration of 0.5-3.0% can induce general anaesthesia in 7 to 10 minutes, with analgesia, muscle relaxation, and loss of consciousness.

5.11 Isoflurane is mildly pungent and may cause coughing, laryngospasm and breath holding in an unconscious individual. Pharyngeal and laryngeal reflexes may be obtunded. Isoflurane is a strong respiratory depressant and may produce hypercapnia.

5.12 Blood pressure is depressed with an initial decrease in systemic vascular resistance, heart rate and cardiac output, and arrhythmias can occur.

Chemical	CAS no	Pictogram	Hazard statements	Precautionary statements
Isoflurane	26675-46-7		H319: Causes serious eye irritation H361: Suspected of damaging fertility or the unborn child H373: repeated or prolonged exposure may cause damage to organs	P280: Wear protective gloves/protective clothing/eye protection/face protection. P260: Do not inhale dust/fumes/gas/mist/vapours/spray.

Kemetyl technical ethanol 96%	CAS no	Pictogram	Hazard statements	Precautionary statements
Ethanol	64-17-5		H225 Highly flammable liquid and vapour H319 Causes serious eye irritation	P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P370 + P378 In case of fire: extinguish with carbon dioxide (CO ₂), foam, powder or water.

6.0 EQUIPMENT AND MAINTENANCE

6.1 Chemical resistant gloves (Kimtech Purple Nitrile Xtra or other suitable gloves)

- 6.2 Regular gloves
- 6.3 Protective coat
- 6.4 Face mask with filter for organic vapours
- 6.5 Safety glasses
- 6.6 Extractor arm fume hood
- 6.7 Fume hood: inspected on a regular basis.
- 6.8 Isoflurane: must be purchased by user
- 6.9 Univentor 410 Anaesthesia machine: the unit should be regularly checked for gas leakages. Service is recommended every 12 months when in use daily and otherwise every 18-24 months, depending on use. The gas outlet filter part number 2423017 is recommended changed at least once a year.
- 6.10 Univentor 2010 scavenger unit: do not use ethanol - this might damage the unit and interfere with the sensor.
- 6.11 Fluovac anaesthetizing system: must be cleaned regularly. Pay attention to the gap between the inner and outer tubes of the mask, as hair could accumulate here and impair the scavenging efficiency of the unit.
- 6.12 Harvard Tec3 Cagemount vaporizer system: follow manual on page 12-15 in this SOP.
- 6.13 Sponges
- 6.14 Cadaver bags, garbage bags
- 6.15 Containers for hazardous waste
- 6.16 Paper towels
- 6.17 Spray bottle with soapy water and 70 % ethanol

7.0 HISTORY OF EDITING

- 7.1 Created 18.11.2020 by Katarzyna Zelewska. Completed 12.01.2021 by Frøydis Kilmer.
- 7.2 Revised 02.08.2023, changed from univentor/scavenger to minivac and vaporizer (Helene Tandberg)

8.0 REFERENCES

- 8.1 Safety Data Sheet, Baxter, Isoflurane https://esirius.uwo.ca/eSirius3g/attachment/2018-178_1_0001_Isoflurane%20Baxter%20MSDS%202016.pdf
- 8.2 [410-univentor-anesthesia-manual-.pdf \(uio.no\)](#)
- 8.3 [2010-scavenger-manual.pdf \(uio.no\)](#)
- 8.4 [Fluovac Anesthetizing System User's Manual \(uio.no\)](#)
- 8.5 [Survival Surgery Procedures: Rodents \(jhu.edu\)](#)
- 8.6 [Felleskatalogen Isofloran](#)
- 8.7 [Minivac Manual](#)
- 8.8 [Vaporizer manual](#)

Romplan ny dyreavdeling

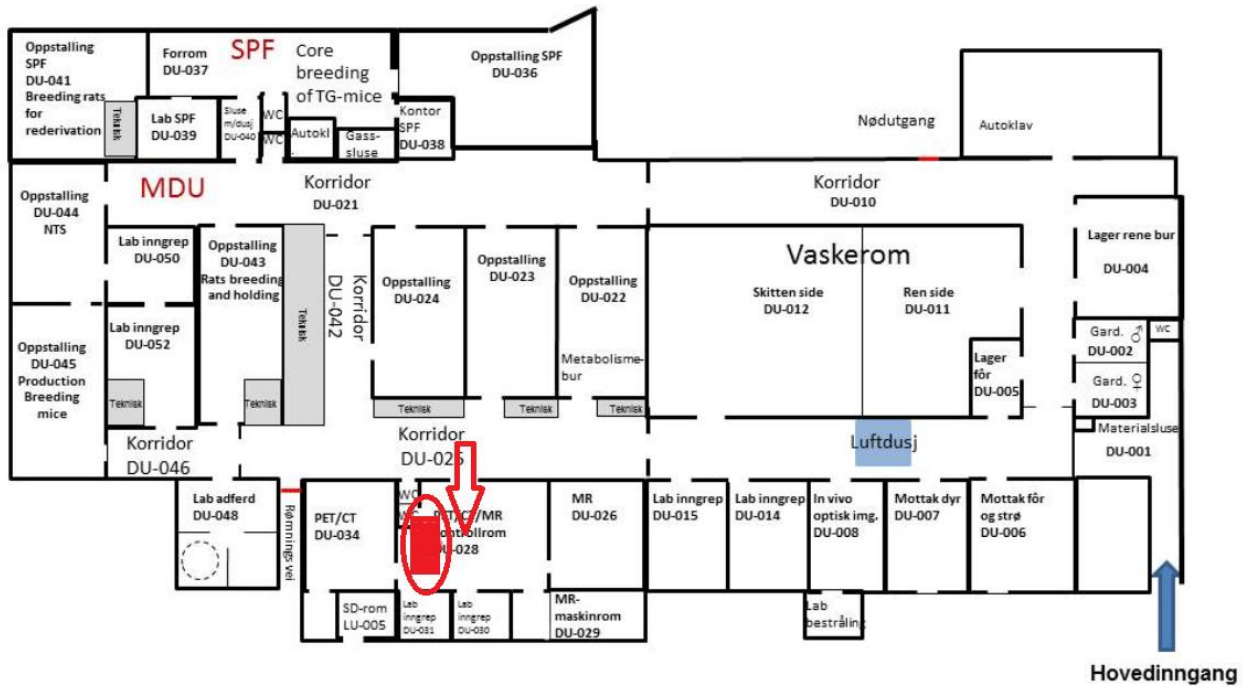


Illustration 1: Location of chemical cabinet



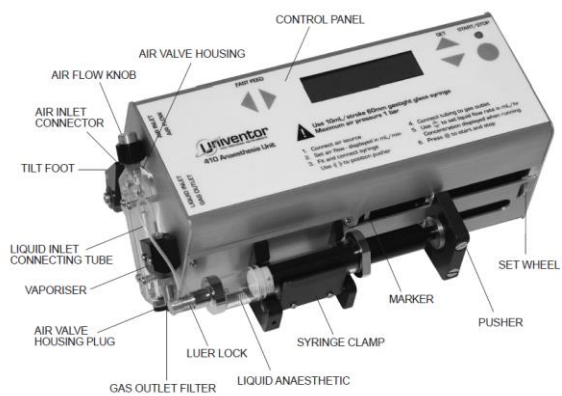
Picture 1: Extractor arm fume hood

<https://makitech.no/met-1000-50-avtrekksarm-tak>



Picture 2: Fume hood

<https://kilab.no/produkter/kategori/avtrekkskap/>



Picture 3: Univentor 410 anaesthesia machine



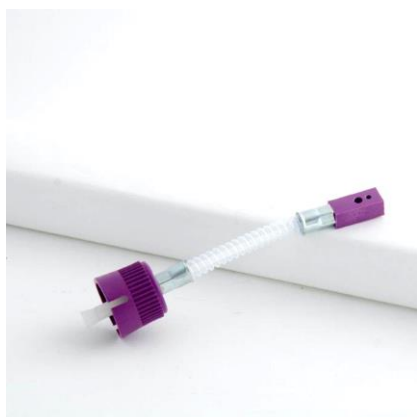
Picture 4: Univentor 2010 scavenger unit



Picture 5: Fluovac anaesthetizing system



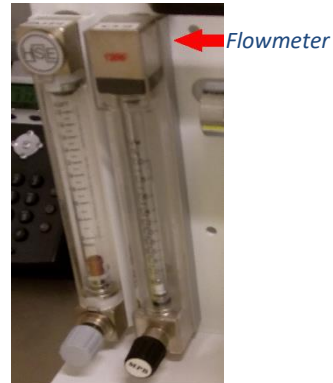
Picture 6: Harvard Tec3 Cagemount vaporizer



Picture 7: Key adapter

How to start and use the anesthesia device (DU-50, 52 and 15):

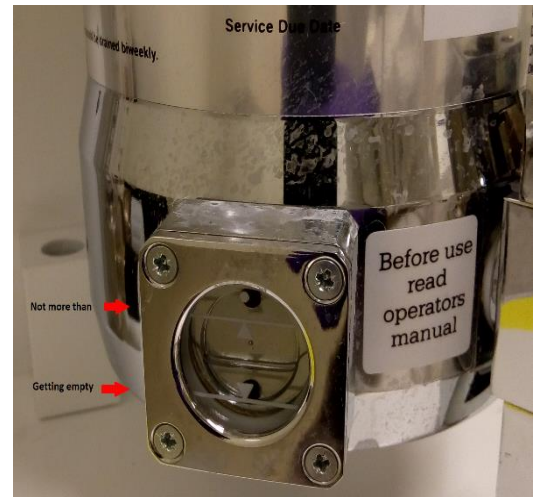
1. On the bench it says Oxygen, turn the valve horizontally, see picture. Turn the flow adjust knob until it says approximately 2 on the barometer.
2. Turn on the oxygen flowmeter on this should stay at about 1,2L.



3. Make sure it is enough isoflurane for the procedure. There is two levels. Do not fill more than the highest level, and it should not be below the lowest level (see pic).



<- Remove the cap here, use the adapter and tighten it with the screw before filling. Put the cap back in when done, and tighten the screw.



4. Turn the minivac on. But if the mice wake should turn it off again, since this might the sedation.



up you help with

5. Turn the vaporizer on. Make sure the flow is heading to the chamber. When the mouse is asleep, turn it to "chamber and mask" and place the mouse on the mask.

The vaporizer level might vary on strain and analgesia. **You can try having the vaporizer on 5% in the beginning to fill the chamber then turn down to 2% when the mice is asleep.** On mask, they need less isoflurane so watch their breathing and check reflexes and adjust the % for what is needed.

PAGE 2

To turn off:

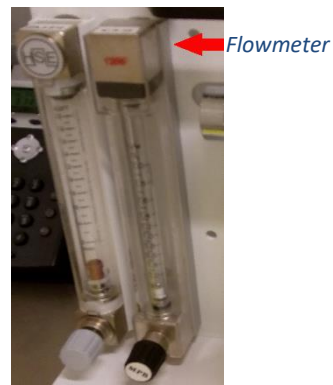
1. Stop the isoflurane
2. Turn off the oxygen on the flowmeter (place the orb on the bottom without force)
3. Turn off the oxygen coming from the bench. First turn the knob (turn it to the left) until it stops, then turn the valve so it is facing vertically (see picture from page1).
If the barometer don't fall to 0 you can open the flowmeter a little. The cause for this is because there is still oxygen in the hose. Remember to turn the flowmeter off again.
4. Empty the transparent chamber and clean it with soap. Leave the chamber cover a bit open so it can dry up.
5. Turn off the MiniVac, I recommend to measure the weight of the filter after each use. When completely new a filter will weigh about 1200g, and it should not exceed 1400g (it tells you on the box as well).
6. Clean the bench with soap and ethanol. Thank you 😊

Nice to know:

- You can always fill more isoflurane; just remember to turn the vaporizer down to 0 before adding.
- Only use Soap on the plastic chamber, it does not like ethanol.
- The amount of bars from the device on the bench does not really matter since you adjust the airflow from the flowmeter. As long as you have enough flow coming in off course.
- The more oxygen you give the animals will also receive more gas, so make sure you balance the amount. Watch the mouse breathing and reflexes to see if it is too deep or too light sleep.

How to start and use the anesthesia device (DU-14):

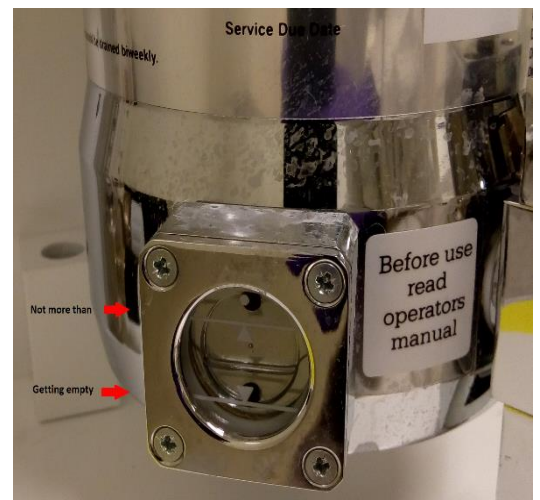
6. Measure the weight of the filter before beginning the procedure. When completely new a filter will weigh about 1200g, and it should not exceed 1400g. (see picture →)
7. On the bench it says Oxygen, turn the valve horizontally, see picture. Turn the flow adjust knob until it says approximately 2 on the barometer.
8. Turn on the oxygen flowmeter, this should stay at about 1,2L.



9. Make sure it has enough isoflurane for the procedure. There is two levels. Do not fill more than the highest level, and it should not be below the lowest level (see picture →).



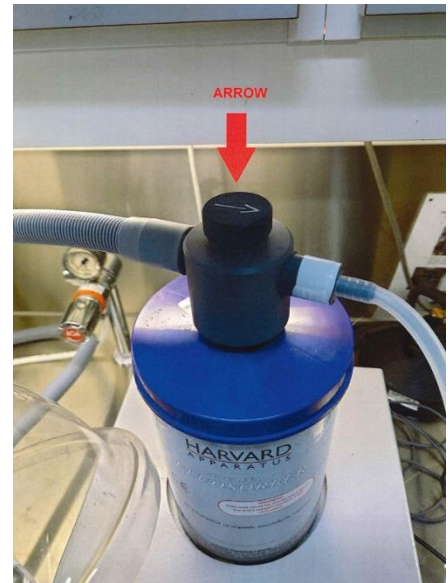
← Remove the cap here, use the adapter and tighten it with the screw before filling. Put the cap back in when done, and **tighten the screw.**



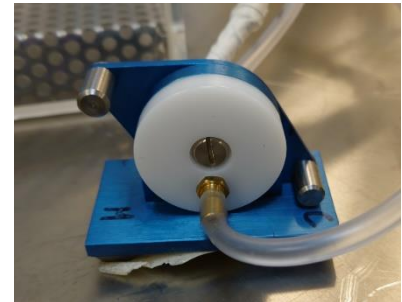
10. Turn the fluovac on
(see picture →)



11. Make sure the flow is heading to the chamber; then set the white arrow, which is on top of the scavenger, to point toward the chamber. When the mouse is asleep, turn it to "mask", and switch the white arrow towards the mask; then place the mouse on the mask. (see picture →)



12. You can either choose to work with mask or chamber. Remember to switch to what you want to use. (See picture of correct choosing for chamber →)



The vaporizer level might vary on strain and analgesia. You can try turn the vaporizer to 3% in the beginning to fill the chamber then turn down to 2% when the mice is asleep. On mask, they need less isoflurane so try 2%, watch their breathing and check reflexes and adjust the % for what is needed.

To turn off:

7. Stop the isoflurane
8. Turn off the oxygen coming from the bench. First turn the knob (turn it to the left) until it stops.
9. Turn the flowmeter off.
10. Then turn the valve so it is facing vertically (see picture from page1).
11. Empty the transparent chamber and clean it with soap. Leave the chamber cover a bit open so it can dry.
12. Turn off the fluovac. Measure the weight of the filter after each use. When completely new a filter will weigh about 1200g, and it should not exceed 1400g.
13. Clean the bench with soap and ethanol.

Do not empty the vaporizer for isoflurane; make sure it has enough fluid to the bottom line so it does not dry out.

Thank you 😊