

**Research Animal Standard Operating Procedures (SOP) must meet the following criteria:**

1. Describe procedures or activities involving research animal(s) common to more than one research project.
2. Support the handling and or performance or undertaking of a procedure(s), involving an animal, in the same way on each occasion it is performed.
3. Describe a procedure or activity involving a research animal(s) undertaken by more than one person; and
4. Describe a procedure or activity involving a research animal(s) that will be undertaken in more than one location.

<b>Name of Procedure</b>	Isoflurane Anaesthesia using an induction chamber	
<b>Species</b>	Mouse and rat	
<b>ACEC</b>	<b>Reference</b>	SOP#6 – Mar 23 - Gaseous anaesthesia (Isoflurane) using chamber Induction
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	<b>Version</b>	1.4
	<b>Date approved</b>	24 March 2023
	<b>Date for review</b>	24 March 2026
	<b>Procedure classification</b>	3
<b>Ethical considerations</b>	<ol style="list-style-type: none"> <li>1. Respect for animals must underpin all decisions and actions involving the care and use of animals for scientific purposes.</li> <li>2. The procedure must be performed according to current best practice to support the wellbeing of the animal.</li> <li>3. Persons performing this procedure must be competent in the procedure or be under the direct supervision of someone who is competent.</li> </ol>	

## Details

### 1. Equipment

#### 1. **Equipment:**

- 1.1 Anaesthetic machine including isoflurane anaesthetic vaporiser.
- 1.2 Anaesthetic chamber with delivery port and scavenge port
- 1.3 Scavenging system, including scavenge device or inlet and tubing.
- 1.4 Medical grade oxygen cylinder (if no access to piped oxygen), regulator and oxygen tubing
- 1.5 Isoflurane
- 1.6 If animal will be maintained under isoflurane anaesthesia with a face mask- Anaesthetic breathing circuit such as paediatric Bain coaxial circuit or Ayres T-piece and rebreathing bag with appropriately sized face mask

### 2. Method

**NOTE:** Only staff trained in the safe operation of the anaesthetic machine are permitted to use the machine. **Training can be obtained from the Animal Welfare Officer.**

#### 2.1 SETTING UP THE APPARATUS

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- 1.1.1 Check that the oxygen is connected at the wall outlet. If you do not have access to piped oxygen and are using a cylinder, check that it is connected turned on and that there is sufficient gas in the cylinder.
- 1.1.2 Add Isoflurane to the vaporiser on the anaesthetic machine using the key filler until it reaches the “fill line” in the clear glass indicator compartment. Replace the plug and screw in firmly.
- 1.1.3 Ensure that the delivery tubing to the anaesthetic chamber is connected to the oxygen/ isoflurane gas outlet on the anaesthetic machine.
- 1.1.4 Set up the gas scavenging system by attaching the scavenge tubing to the scavenge inlet on the wall if present, or to the activated charcoal scavenge canister. If using an activated charcoal scavenge canister, weigh before use and follow the manufacturer’s instructions to ensure that the maximum isoflurane has not already been absorbed by the canister. Replace if necessary.



## 1.1 CHAMBER INDUCTION

- 1.1.1 The oxygen cylinder should be turned on already (see 2.1.1). The oxygen flow rate is then controlled by the Oxygen flow meter on the anaesthetic machine.
- 1.1.2 Put the animal into the chamber before turning on the oxygen or adding anaesthetic gas.
- 1.1.3 Turn on the oxygen at the oxygen flow meter. Use a high oxygen flow rate to ensure that the chamber fills quickly e.g. a 12 litre chamber will fill in three minutes if a flow rate of 4 litres per minute is used. The oxygen flow rate is adjusted using the white knob in front of the Oxygen meter. The flow is measured from the top of the cylinder in the flow meter (or centre of the silver ball if present)
- 1.1.4 Turn the release dial on top of the vaporiser to set Isoflurane concentration to 4 or 5% for induction. Hold down the button and turn the dial clockwise to the desired number.
- 1.1.5 Oxygen carrying Isoflurane should now be entering and filling the anaesthetic chamber and departing through the scavenge system.
- 1.1.6 **Scavenge gas from the chamber before opening the lid to prevent human exposure to concentrated gas.** This is done by turning off the isoflurane and allowing pure oxygen to enter the chamber for a time sufficient to completely fill the chamber with 100% oxygen. The oxygen flush valve on the anaesthetic machine can also be used to flush the anaesthetic chamber with pure oxygen.



## 1.2 MAINTENANCE OF ANAESTHESIA

- 1.2.1 Remove the animal from the chamber when it loses its righting reflex (can no longer stand). This can take up to several minutes. Check the state of the animals during the process as if they are left in the anaesthetic chamber for too long, they can stop breathing completely.
- 1.2.2 Replace the anaesthetic chamber with the Bains or T piece breathing circuit and face mask, attached to the anaesthetic machine.
- 1.2.3 Attach scavenge tubing to the outlet of the breathing circuit.
- 1.2.4 Place the animal on a heating pad and maintain anaesthesia with a close fitting face mask using 1 to 2.5% Isoflurane,
- 1.2.5 Eye lubricant (e.g. Lacrilube) should be applied to the eyes to protect the cornea.
- 1.2.6 Assess the animal's breathing and reflexes frequently and change the Isoflurane concentration to modify the depth of anaesthesia to suit the procedure. 2-3% should be the maximum concentration needed for a surgical level anaesthesia.
- 1.2.7 Turn the oxygen flow rate to 1 litre per minute.

## 3. Drug Details

Give details of the anaesthetic agent(s) and technique to be used. Include details of sedatives or tranquilisers.

Drug name (generic name, not trade name)	Dose Rate (mg/kg body weight)	Route	Timing of administration, and frequency
Isoflurane	Varies from 1-5% in oxygen (1-3 l/min)	Inhalation	Immediately prior to procedure requiring anaesthetised animal

## 4. Monitoring during anaesthesia

What clinical or physiological criteria will be used to monitor the depth of anaesthesia and general well-being of the animal during the anaesthesia? Please attach copies of any forms used for anaesthetic monitoring.

- Respiratory frequency will be monitored to ensure slow constant breathing
- Heart rate will be palpated to monitor rate and regularity of beats.
- Colour of extremities (nose, feet), mucous membranes of nose and mouth) will be monitored to ensure that they maintain a pink colour
- The adequacy of the depth of anaesthesia will be checked intermittently using lack or reflexes such as the withdrawal reflex (flexion of the leg following a firm pinch of the paw or interdigital skin) or the palpebral reflex (in response to stroking the eyelids).
- Observations will be recorded on the attached anaesthetic monitoring record sheet.



