

## SOP for Isoflurane Drop Method

1. All isoflurane usage for anesthesia requires documentation; labs may use the [Isoflurane Drop Method Log Template](#) or create a similar log.
2. Pre-charge the sealed container by placing sufficient gauze/absorbent material on the bottom of the chamber, below the protective layer (i.e., perforated platform), ensuring the animal cannot come in direct contact with the solution. Add approximately 5 ml (1 to 2 capfuls) of isoflurane liquid to the gauze. Close the lid and wait 5 minutes for the liquid to form a volatile gas within the chamber. (Note: the actual amount of isoflurane needed may vary as the amount depends on the size of the chamber.)
3. Remove the lid of the container and quickly place the animals onto the protective layer within the chamber. Immediately close the lid. (For perforated platforms, ensure that the perforations are not large enough for the animal to crawl or reach through and contact the isoflurane-soaked material.)
4. The animals will initially exhibit an excitatory phase, after which they become anesthetized typically within 2-5 minutes. Neonates must remain in the chamber for at least five (5) minutes.
5. When animals are recumbent and breathing is slow and steady, remove them from the container and replace the lid.
6. For painful procedures, a noxious stimulus (i.e., toe pinch) on all four paws is required and documentation recorded. The absence of a withdrawal reaction in all four paws indicates appropriate anesthetic depth and the procedure may begin.
  - **Note:** Isoflurane is highly volatile and animals will quickly regain consciousness once removed from the chamber; thus, it is imperative to perform and complete the procedure as quickly as possible.
7. Once the procedure is complete, recover the animal in a clean cage, bedding covered if present, until the mouse/rat is fully ambulatory.
8. If utilizing the drop method for euthanasia, the animal should be left inside the container until cessation of breathing is observed (neonates may require longer exposure). Remove the animal quickly and perform a secondary physical method.
  - **Note: All gas euthanasia must be followed with a secondary physical method unless the protocols has an approved exception.**