

Proper Steps for CO₂-Euthanasia of Rodents with a Precisely-Regulated Gas Flow

For a humane death, the CO₂ flow rate must equal 30-70% of chamber volume per minute.

Never place animals into chambers prefilled with CO₂!

- 1) You must be trained before you perform this procedure. If you have questions, contact DCM-Training@Downstate.edu
- 2) Read this entire protocol and check your setup before starting:
 - A clear euthanasia chamber or home cage that lets you see the animals' breathing motion
 - Sufficient compressed, medical-grade CO₂ (cylinder pressure at least 250 psi)
 - A pressure regulator and a flow meter, both functional, between gas cylinder and chamber
- 3) Look up or calculate the correct flow rate for the specific size of your euthanasia chamber:

$$\text{CO}_2 \text{ flow rate in } \frac{\text{L}}{\text{min}} = \frac{\text{height in cm} \times \text{width in cm} \times \text{length in cm}}{1000} \times \frac{0.5}{\text{min}}$$

Mark the rate on the flow meter with a piece of tape and record it here:

Chamber type: _____ CO₂ flow rate: _____ L/min

- 4) Place the mice or rats into the chamber and close it. Alternatively, attach the tubing from the flow meter to the home-cage inlet. In order to minimize distress, animals from separate cages should not be comingled.
- 5) Open the cylinder valve to release CO₂ into the chamber and adjust the flow to the correct rate from point 3. Do not leave the animals in the chamber unattended—you must remain nearby at all times.
- 6) **For rodents >10 days of age:** keep the chamber closed and the CO₂ flowing until you see that respiration has ceased in all animals (typically 3 to 5 mins).
- 7) **For rodents <10 days of age:** keep the chamber closed and the CO₂ flowing for at least 50 minutes.
- 8) Stop the CO₂ delivery by closing the valve or flow meter.
- 9) Ensure that all animals are dead by using a secondary, physical method of euthanasia as approved in your IACUC protocol, such as cervical dislocation, decapitation, removal of vital organs, or bilateral pneumothorax.
- 10) Bag and return carcasses to DCM for proper disposal.
- 11) Before the next group of animals, refill the chamber with room air by turning it on its side to let the heavier CO₂ flow out. Disinfect the chamber and wipe it clean if it is soiled.
- 12) After the last group of animals, disinfect and clean the chamber and close the cylinder valve. If the gas cylinder is (almost) empty, make sure that it is replaced before the next user.

