

# LOYOLA CMF

## CO2 CHAMBER INSTRUCTIONS

**DO NOT USE FOR NEONATAL ANIMALS LESS THAN 10 DAYS OF AGE.**

**DO NOT USE THE CHAMBER IN AN ANIMAL ROOM UNLESS APPROVED BY THE IACUC**

**DO NOT MIX SPECIES**

**DO NOT PRECHARGE CHAMBER**

**TURN CO2 OFF AT SPIGOT WHEN FINISHED**

- 1) The euthanasia chamber should allow ready visibility of the animals. Do not overcrowd the chamber: all animals in the chamber must be able to make normal postural adjustments.
- 2) Without pre-charging, place the animal(s) in the chamber or place lid on home cage (without filter top). If using the home cage, cover water/vent grommet on the end with the reusable black sticky mat provided on the wall (return to wall after use).
- 3) Ensure CO2 hose is connected to chamber or chamber lid before turning on the CO2.
- 4) Turn the flow meter to appropriate 30% fill rate (see chart below). Filling at a rate above 70% is prohibited (see chart below). Contact a CMF staff member if you notice the animals exhibiting undue stress or discomfort during the euthanasia process.
- 5) Expected time to unconsciousness is usually within 2 to 3 minutes. Observe each rodent for lack of respiration and faded eye color. Maintain CO2 flow for a minimum of 1 minute after respiration ceases. If both signs are observed, then remove the rodents from the cage; otherwise continue exposing them to CO2. If unconsciousness has not yet occurred within 2 to 3 minutes, the chamber fill rate should be checked. The system should also be examined for a defective flow meter, absence of CO2 supply, and/or leaks. Appropriate CO2 concentrations and exposure times will prevent unintentional recovery.
- 6) Upon completion of the procedure, death must be confirmed by an appropriate method, such as a secondary euthanasia method or by ascertaining cardiac and respiratory arrest or noting an animal's fixed and dilated pupils.
- 7) Euthanizing in the home cage is preferable to prevent added stress. If this is not feasible, the provided chamber may be used. When euthanizing successive groups of animals using the same cage/container, the euthanizing container should be cleaned between uses to remove the potential distress secondary to remaining pheromones, etc. Always clean the chamber when finished. The soiled home cage may be left in the dirty cage room.

CHAMBER TYPE	30% FILL RATE	70% FILL RATE*	COMMENTS
CMF mouse cage	2L/min	4.5L/min	Cover holes if possible
CMF rat cage	5.5L/min	12.5L/min	Cover holes if possible
Cancer Center mouse cage	2L/min	4.5L/min	Cover holes if possible

\*do not exceed 70% fill rate