MOUSE / RAT EUTHANASIA INSTRUCTIONS

In-House CO₂ Setup

For more detailed step-by-step instructions, please refer to the Guidelines for Rats & Mice Euthanasia Procedures for Investigative Personnel (az.research.umich.edu/node/1016)
REMEMBER, IF CONSOLIDATING ANIMALS FOR EUTHANASIA:

**No more than** 10 mice in a standard mouse (#1) cage or 25 mice in a large mouse / small rat (#3) cage.

Follow standard housing densities when consolidating other rodents (e.g. rats) for euthanasia.

Provide ample space for animals to move and assume normal postures if using your own euthanizing chamber.

Start the procedure **no more than 10 minutes** after the animals have been consolidated.
The flow meter (1) should be at zero.

The supply valve (2) and flow meter (3) should be turned off (all the way clockwise).
Insert the CO$_2$ hose through the water bottle grommet on the cage lid

OR

through the lixithole in the back of the cage
STEP 03

Turn supply valve (1) to the left or counterclockwise to turn on the CO₂
Adjust CO₂ flow into the cage by turning regulator knob (1) slowly

Until the gauge (floating black ball) (2) reaches the desired color line

BLACK is for RAT #3 cages
GREEN is for LARGE MOUSE #3 cages
BLUE is for SMALL MOUSE #1 cages

INSET
Allow gas to flow until animals are no longer moving or breathing

You **MUST** remain with your animals until death is confirmed by a secondary means.
Turn off the CO\textsubscript{2} supply valve (1)

Note that gauge (2) is falling back to zero now
Gauge should now be set at zero with no more CO₂ flowing.

After gauges reach zero, ensure flow meter valve (1) is shut off by turning it clockwise until snug.

Do NOT overtighten.
Check your protocol for approved secondary euthanasia method

ALWAYS perform a secondary, physical method to ensure euthanasia is effective
After you have confirmed death by a secondary, physical means:

Put carcass in a glove or opaque bag.

Label with PI name, date, and name of individual conducting euthanasia.
Place in appropriate bin within carcass cooler
We want to acknowledge that the ethical cost of using animals in research may cause some individuals emotional distress, especially if they perform euthanasia as part of their occupational/research duties.

If you think you may be struggling with what is commonly referred to as “compassion fatigue,” there are resources that can help.

Visit animalcare.umich.edu/cap or email CompassionFatigue@umich.edu to learn more about our Compassion Awareness Project.