

School of Biomedical Sciences – Standard Operating Procedure  
**Integrated Physiology Facility (IPF), MacGregor Building (64) Room 445**

15th December 2021

## **Hypoxic Chamber Pro Ox 110**

### **I. Objective**

Provide an altered oxygen environment for animals.

### **II. Comments and Recommendations**

- This procedure has been written with specific reference to the Integrated Physiology Facility (IPF), the Hypoxia chamber and Pro Ox 110 may only be used at this facility by IPF approved experienced operators
- Use of the Hypoxia chamber and Pro Ox 110 and any variation to this SOP must be described in a relevant animal ethics application
- Users should further read and understand the associated Risk Assessments prior to operation: 21762 Hypoxia Chamber; 3657 UQBR Handling and restraint of laboratory animals; 3940 Handling rats and mice (available on the [UQSafe](#) website)
- This procedure requires an understanding of gas handling procedures. It is recommended that all users undertake the [Compressed Gases Safety online training](#) module via the UQ Staff [Health and Safety Training and Induction](#) website
- Equipment/software failures and animal escapes need to be reported to the animal facility manager immediately
- All incidents/injuries should be reported via [UQSafe](#) online
- Spills must be cleaned up immediately
- Use of the hypoxia chamber involves mouse handling and appropriate care should be taken, refer to [LAB 006 Handling and restraint in mice and neonates](#), and [LAB 039 Handling and Restraint in Rats and Neonates](#)
- Wild type and genetically modified animals must be transported to equipment as per OGTR guidelines and [LAB 003 Transportation of Laboratory Rodents](#)
- The IPF is a shared space with unknown commensal microbial status. Once transported to a shared space it is often not possible, for biosecurity reasons, to return rodents to their original animal facility. Arrangements for transportation and ongoing care of experimental animals must be made with relevant animal facility managers when planning projects that aim to use a shared facility

### **III. Equipment**

- Minimum Personal Protective Equipment (PPE) consists of gloves, gown, closed in shoes, eye protection and face mask. Additional PPE may be required based on added risk e.g., working with infectious animals (P2 fitted mask and viral gown)
  - Hypoxia chamber and Pro Ox 110
  - IVC Tecniplast cages and open top wire lids
- Three IVC cages with up to four mice in each can be housed in the hypoxia chamber for the duration of each experiment*
- Purachip bedding, Envirodry nesting material

- Mouse pellets

#### IV. Preparation

- The low level of oxygen (<21%) in hypoxic chambers is maintained by automated addition of nitrogen gas. If tanks are allowed to become empty oxygen levels would normalize compromising the quality of your experiment and results
- Based on previous experiments, to maintain a constant 10%-12% oxygen level, nitrogen tanks need to be replaced every 48 – 72 hrs
- Oxygen levels are measured and adjusted by the Pro Ox 110 controller
- All operations are executed by the Pro Ox 110 controller
- A gas regulator (2500 PSI input, 0-60 PSI output) set at a maximum of 25 PSI is required to ensure no damage occurs to the Pro Ox 110
- Prior to use ensure the Pro Ox 110 and chamber have been calibrated by the IPF manager
- Check booking date/s and time/s on PPMS
- All animal arrivals/departures and euthanasia's must be recorded on the Mosaic movement sheet available in the animal facility

#### V. Procedure

##### To start:

1. Ensure the correct gas cylinder as attached (N<sub>2</sub> for low O<sub>2</sub> environment and O<sub>2</sub> for high O<sub>2</sub> environment)
2. Turn the gas on and off at the cylinder, the correct pressure is set on the regulator
3. Switch the unit on at the wall, 2 switches
4. Check the sensor is in the correct mode (work or program mode). The mode must be changed when swapping from high to low oxygen
  - Enter program mode by simultaneously holding the up and down keys for 3 sec
  - Press down arrow to get to level 1
  - Press and hold the \* key and use the arrows to move to level 3
  - Use the up arrow to reach rEU.D and select output modes (direct/reverse). The display will flash between 1d2r for direct mode (i.e. low oxygen) and 1r2r for reverse mode (i.e. high oxygen)
  - Change the mode using the \* and arrow keys
  - Exit by pressing and holding up and down keys for 3 sec
5. Check the sensor has the correct burn-out protection setting. This is changed when swapping modes
  - Use up arrow to reach burn in level 3. The display will flash between dn.SC which is selected for direct mode (i.e. low oxygen) and up.SC for reverse mode (i.e. high oxygen)
  - Change the burn using the \* and up keys
  - Exit by pressing and holding the up and down keys
6. Check the set point is at the desired level by pressing and holding the \* key
7. Change the set point, if necessary, by using the \* key and the up and down arrows
8. Close the door to the chamber
9. Turn the gas 'on' on the front of the unit, you should be able to hear gas hissing

##### To Finish:

1. Open the chamber
2. Turn the gas 'off' on the front of the unit
3. Turn the gas off at the cylinder (not the regulator)
4. Turn unit off at the wall

#### **To Check Calibrations:**

- The sensor and unit must have the calibrations checked weekly. The sensor sensitivity drifts downwards with time and will need to be adjusted. The procedure is different for high and low oxygen environments

#### **Calibrations for low oxygen:**

1. Turn system on as above
2. Turn gas 'off' on the front of unit
3. Remove sensor tip from animal chamber and place in calibration chamber
4. Open brass bleed valve to flood calibration chamber with nitrogen. Should get 0% oxygen reading on front of unit. If not continue, with steps 5 and 6 below
5. Enter program mode and navigate to level 3 → zero. Raise the setting if the reading was too low using the \* and up arrow or lower it using the \* and down arrow. Exit program mode by pressing up and down key for 3 seconds
6. Try again using the calibration chamber and continue to adjust until you achieve a reading of 0% and turn off bleed valve
7. Place the sensor in room air. Reading should be 21%. If not continue with 8 and 9 below.
8. Enter program mode and go to level 3 → span. Raise the setting if the reading was too low using the \* and up arrow or lower it using the \* and down arrow
9. Try again in room air and continue to adjust until you achieve 21%
10. Replace sensor into animal chamber
11. Turn on gas on front of unit to start normal operations

#### **Calibrations for High oxygen:**

1. Turn system on as above
2. Turn gas 'off' on the front of unit
3. Remove sensor from animal chamber and place in room air. Reading should be 21%. If not continue with steps 4 and 5 below
4. Enter program mode and go to level 3 → zero. Raise the setting if the reading was too low using the \* and up arrow or lower it using the \* and down arrow. Exit program mode by holding the up and down keys for 3 seconds
5. Try again in room air and continue to adjust until you achieve 21%
6. Place the sensor tip in the calibration chamber, open the brass bleed valve to flood the chamber with oxygen
7. Reading should be 100%. If not continue with steps 8 and 9
8. Enter program mode and go to level 3 → span. Raise the setting if the reading was too low using the \* and up arrow or lower it using the \* and down arrow

9. Try again using the calibration chamber and continue to adjust until you achieve 100% then turn off bleed valve
10. Replace sensor in animal chamber
11. Turn on gas on front of unit to start normal operations