

University Animal Care Committee Standard Operating Procedure		
Document No: 10.27	Subject: Hypoxic Studies in Rats	
Date Issued: July 22 nd , 2024	Revision: Original	Page No: 1

Location: Queen's University

Responsibility: Principal Investigators, Research Staff, Veterinary Staff

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe the method of hypoxic studies in rats.

- 1. Introduction and Definitions:** The purpose of this Standard Operating Procedure (SOP) is to describe the method used for inducing pulmonary hypertension by exposing mice to hypoxia.

Abbreviations: Animal Care Services **ACS**, Principal Investigator **PI**, subcutaneous **SC**, intravenous **IV**, intraperitoneal **IP**, intramuscular **IM**, per os **PO**, per rectum **PR**

2. Materials:

- ProOx Sensor
 - Calibrated to maintain the level of oxygen at 10% O₂ within the chamber. The alarm should be calibrated to allow for +/- range of 2.0% which, if exceeded, will cause the alarm to sound and the gas to be turned off.
- CO₂ Sensor
 - Measurements of CO₂, O₂%, temperature, and humidity will be recorded daily.
- CO₂ Absorbable Granules (soda lime)
- Desiccant beads (eg. DRIERITE tm)- Anhydrous indicating desiccant or equivalent
- Cages
- Water Bottle
- Nitrogen Gas

3. Procedures:

- Submit a special request for supplies needed (ie. caging, diet, water bottles) and include the study timeline.
 - Rat cage changes will be done on a minimum of twice weekly (Tuesday and Fridays) and if there is any visual condensation.
 - The ProOx sensor will be calibrated and set to maintain an internal oxygen percentage of 10%. The sensor connection will be checked to ensure a secure
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contact with the controller. A loose connection can give an erroneously high oxygen reading, causing the chamber to be flooded with nitrogen, asphyxiating the rats.

- Rats will be transferred into a clean cage with adequate food and water.
- The cage will be placed into the hypoxic chamber.
- The gas will be turned on and will fill the chamber until the ProOx sensor reads between 8-12%.
- Once the chamber is steady at the set point, the alarm will be turned on to prevent the chamber from deviating from the desired oxygen percentage (+/- 2%).
- The O₂%, CO₂ level, temperature and humidity will be recorded daily.
- The rats will be monitored daily by looking into the chamber and assessing overall wellness.
- The chamber will be opened, and rats will be transferred into a new, clean cage weekly. The gas and alarm will be turned off when the chamber is opened.
- The new cage will be placed back into the chamber, and the gas will be turned on.
- Each time the chamber is opened, the silenced alarm must be reset once the internal O₂% has stabilized.

4. Complications:

- Failure of the sensor may result in a reading of internal O₂% $\leq 8\%$ or $\geq 12\%$. At this point the alarm will sound. For the low O₂ alarm, gas will automatically shut off. Research staff will assess the sensor and nitrogen tank and determine the reason for failure before turning the gas back on.
 - The absorbable granules will be monitored for a colour change from white to purple as a measure of CO₂ absorption within the chamber. If the scrub is found to indicate high CO₂ absorption (purple colour) this will result in the chamber being opened and the scrub being changed. An additional container of absorbable granules can be added to help maintain the level of CO₂ within the chamber.
 - The desiccant will be monitored for a colour change as per the manufacturers instructions as a measure of internal humidity. Change of colour will result in the chamber being opened and the desiccant being changed.
 - Cages will be monitored to ensure that the rats have adequate food, water and they will also be monitored for water leakage and flooding. For any of these events, the chamber will be opened, and the specific issues resolved.
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References: Dr. Archer and Dr. Ormiston Laboratories

SOP Revision History:

Date	New Version