LAB_010 Euthanasia – Hypothermia and Rapid Freezing in Mice and Rat Embryos and Neonates (Expiry: Sept 2028)

I. OBJECTIVE

To describe the standard, safe and humane euthanasia of embryonic and neonatal mice and rats via hypothermia and rapid freezing, also reflecting the procedure used to train workers across UQ within UQBR.

NOTE

- **When citing this SOP the document *LAB_100 Euthanasia Methods in Rats and Mice* also forms part of this SOP.**
- **You must also describe your chosen anaesthetic technique (or quote the relevant SOP you will be following)**
- **The use of (*) indicates this statement is dependent on the facility procedures**
- **The use of (**) indicates this statement is dependent on AEC Approvals**

II. COMMENTS / RECOMMENDATIONS – Refer to LAB_100 Euthanasia Methods in Mice and Rats

Table 1. Methods of euthanasia in rodent embryos and neonates by developmental stage

Method	Embryos	Neonate (P0-P10)
LAB_007 Euthanasia - Cervical Dislocation in Mice and Rats	X	✓
LAB_008 Euthanasia - Carbon Dioxide Asphyxiation in Mice and Rats	X	✓
LAB_009 Euthanasia – Decapitation in Mice and Rats	✓	✓
LAB_010 Specifically Euthanasia via Hypothermia	All Embryonic ages	<P10
LAB_010 Specifically Euthanasia via Rapid Freezing under anaesthesia or hypothermia	Following anaesthesia or hypothermia acceptable from E15 – Birth.	Following anaesthesia or hypothermia P0-P4.
LAB_010 Specifically Euthanasia via Rapid Freezing	<E15 Acceptable	X
LAB_011 Euthanasia - Lethal Injection in Mice and Rats	✓	✓

III. SAFETY AND COMPLIANCE – Refer to LAB_100 Euthanasia Methods in Mice and Rats

IV. TRAINING CONSIDERATIONS – Refer to LAB_100 Euthanasia Methods in Mice and Rats

V. EQUIPMENT

- PPE*
- Cadaver bag*
- Bench protector material
- CO2 or Anaesthesia Equipment**
- Hypothermia source (4°C) *This may be a conducting cold plate or ice slurry (4°C), commercial freezer (-10°C), liquid CO_2 (-56.6°C), dry ice (-78.5°C), or liquid nitrogen (-210°C).*

VI. PREPARATION

- Check AEC approvals to ensure that the correct procedure and personnel are approved for the planned work
- Check cage cards, animal records and identification to ensure the correct animals are euthanised.

VII. PROCEDURE

Hypothermia can be performed on embryos at any age, and neonates that are <P10.

1. Once the dam has been euthanised, remove the intact uterus (with the embryos inside) and place directly into the cold media.
   The embryos are unconscious in utero and hypoxia does not invoke a response. The uterus will also act as a barrier between the skin and the cold source.

2. If the embryos are removed from the uterus or for neonates (P0-10), wrap the pup in paper towel or inside the finger of a glove and place it onto the cold source to begin reducing the body temperature to induce unconsciousness.
   Direct contact with the cold source must be avoided as it can cause tissue damage and potential pain.

3. Once loss of movement is observed, it should be followed up with a secondary method.
   'There are no data to support the use of hypothermia as a single method, and it should be followed with a secondary method following loss of movement' (AVMA 2020).

Rapid Freezing can be performed on embryos at any stage, and neonates < 4 days under the conditions noted below.

- Rapid freezing alone can be performed in embryos <E15
- If following anaesthesia or hypothermia, rapid freezing can be performed in embryos E15 to birth and neonates P0 to P4..

1. For embryos at E15 to birth, or neonates <P4, anaesthetise via hypothermia or inhalable chemical anaesthetics**.

2. Wrap the embryo/neonate in aluminium foil and lower into liquid nitrogen for rapid freezing.

VIII. BIBLIOGRAPHY – Refer to LAB_100 Euthanasia Methods in Mice and Rats

Version #	Reviewing AEC (note: all other relevant AECs ratify the approval)	AEC Review Date	Outcome
3.1	Laboratory Biomedicine AEC	July 2025	Approved with required changes (admin)
3.1	Molecular Biosciences and Health Sciences AECs	July 2025	Approved
3.1	Anatomical Biosciences AEC	July 2025	Changes required (admin)
3.2	Anatomical Biosciences AEC	Sept 2025	Approved