EMITIMAGING

EMIT ANIMAL AND TISSUE PREPARATION PROCEDURE

Pre-preparation Setup

Dewar

Cooler

Pan

Materials

Whole Mouse (Preferred Method)	**Whole Mouse (Method 2) and Tissues**
Dewar flask	Cooler and Metal pan
Hexanes, ACS grade	Aluminum foil
Dry ice	Dry ice
Clear plastic bags	Clear plastic bags
Sharpie/Permanent marker	Sharpie/Permanent marker

- Euthanize animals using CO_2.
- Begin the freezing procedure immediately following euthanasia.
- It is recommended that all steps are performed in the same room

Freezing Procedures

I. Freezing whole mice – Preferred Method

Note: the preferred method for freezing whole animals is submersion in a cold bath of hexane and dry ice (isopentane may be substituted for hexanes). If the use of hexanes is not permitted in your facility, use Method 2. We **do not** recommend freezing animals in liquid nitrogen as it freezes too quickly and can result in cracks.

Note: Hexanes are volatile and flammable, perform the steps in a fume hood, and wear safety glasses and thermal gloves.

1. Place dry ice in a tall Dewar flask, using enough to cover the bottom.
2. Slowly add the hexanes to the dewar.
   - *Add hexanes to a height that will allow full submersion of the mouse without touching it to the dry ice at the dewar bottom.*
3. Allow the temperature of bath to equilibrate to the dry ice freezing point (-78°C).
   - *Boiling of the hexanes will cease once the bath temperature is equilibrated.*
   - *There should still be dry ice at the bottom of the dewar, add more if needed.*
4. Hold the mouse by the tail and slowly lower it into the cold bath.
   - *The hexanes should not boil over during this process.*
   - *It should take a few minutes to submerge the entire mouse.*
5. Once the mouse is fully submerged, let it remain in the hexanes for 10 additional minutes.
6. Remove the mouse from the bath and store individually in a clearly labeled bag.
7. Place the sample in the freezer and store at -80°C.

II. Freezing whole animals – Method 2:

1. Cover the bottom of a cooler with crushed dry ice.

2. Place an aluminum foil-lined metal pan on the crushed dry ice.
3. Cover the cooler and allow at least 10 minutes for temperature equilibration.
4. Place the mouse on the foil-lined pan, belly side down, with arms and legs extended.
5. Quickly cover the mouse with **powdered** dry ice.
6. Cover the cooler and leave for 45 minutes to allow the mouse to freeze solid.
7. Once frozen, transfer the mouse to a clearly labeled bag and store at -80°C.

III. Freezing tissue specimens

1. Cover the bottom of a cooler with crushed dry ice.
2. Place an aluminum foil-lined metal pan on the crushed dry ice.
3. Cover the cooler and allow at least 10 minutes for temperature equilibration.
4. Place the tissue in the desired conformation/orientation onto the foil-lined pan.
5. Cover the cooler and leave for 5-10 minutes to allow the tissue to freeze in its natural state.
6. Wrap the tissue in the foil that it was chilled on.
    - ***Note:*** *Tissue must be frozen prior to wrapping! Wrapping up the tissue before it is frozen will destroy the anatomical morphology.*
7. Wrap all samples carefully in additional pre-cooled, labeled foil.
8. Place in a pre-chilled, labeled plastic bags.
9. Place the samples in a pre-chilled freezer box and store at -80°C.

If you need any further assistance or have questions, contact info@emitimaging.com