
Preparation of Frozen Tissue Blocks

1.0 Purpose and scope.

Tissue samples may be collected from patients for research purposes. If tissues do not require fixation or are immediately used for preparation of cell suspensions, they must have **rapid** low temperature freezing to maintain the tissue components in as life like a state as possible. Tissues may be placed directly into liquid nitrogen or may be cryoprotected and frozen in liquid nitrogen cooled isopentane.

2.0 COSHH / Health & Safety

Local Health and Safety regulations with regard to the wearing of appropriate PPE and the use of adequate ventilation where appropriate must be adhered to. COSHH forms should be completed for the use of isopentane, risk assessments for the use of liquid nitrogen and also BIOCOSHH assessment of the risk of exposure to unfixed tissues.

Hazard assessment

Isopentane - Highly flammable, has degreasing effect on the skin which may result in inflammation.

Ingestion causes nausea and vomiting and in large quantities may cause drowsiness, dizziness, euphoria, excitation, spasms and in certain circumstances, narcosis.

Liquid nitrogen must be used in a well ventilated area with oxygen level monitors present if necessary.

Toxicity

Isopentane - No evidence of carcinogenic properties or of mutagenic or teratogenic effects.

Storage

Isopentane - Flammable bin.

PPE

Eye protection, nitrile gloves, lab coat, heavy duty insulated gloves for use with liquid nitrogen to protect skin from burns.

Spillages

Isopentane - Shut off all sources of ignition. Mop up spill with paper towel and leave to evaporate in a fume hood. Wash area with copious amounts of water.

Liquid nitrogen – Evacuate area until oxygen levels restored.

Waste disposal

Isopentane – Small amounts of liquid (<10ml) can be left to evaporate in a fume hood. Larger volumes of liquid must be disposed of via an accredited disposal contractor. Store waste in a flammable bin prior to disposal.

Training

Ensure that all staff are familiar with safe handling procedures and have read and understood the COSHH/BioCOSHH assessment.

3.0 Equipment / reagents

Dewar flask
Isopentane
Metal freezing bath
Liquid nitrogen
OCT (cryoprotectant) – optional
Labelled Freezer bags/boxes

4.0 References

- 4.1 HTA
- 4.2 University/Faculty Policy
- 4.3 Department of Cellular Pathology, Royal Victoria Infirmary

5.0 Procedure

1. Pour a small amount of isopentane into a metal freezing bath.
2. Slowly lower the bath into a dewar containing liquid nitrogen. **DO NOT** submerge the bath. Leave to freeze for several minutes.
3. Prepare the tissue blocks as required.
4. Remove the freezing bath from the liquid nitrogen and allow to thaw sufficiently so as to allow complete submersion of the tissue blocks. This can be speeded up by thawing a central well using a spatula. The isopentane should be at its melting point – ensure that there is still some frozen isopentane in the bath.
5. Freeze the tissue by plunging into the isopentane for a few seconds while agitating gently.
6. Blot the excess isopentane from the frozen tissue, on some paper towel before placing into labelled bags/boxes/tubes for storage.
7. Remove the freezing bath to a fume hood to allow the isopentane to evaporate.
8. Amend records accordingly.

6.0 Site Specific Details

Personnel: Staff and students within Uteroplacental Tissue Bank Group,
Institute of Cellular Medicine and Institute of Genetic Medicine

Location: M3066, 3rd Floor Leech Building and G205, 2nd Floor, East Wing, ICfL

Induction: All are provided with a written protocol with safety details. Demonstration is given with continuing supervision as necessary.