Freezing Tissue in OCT using Isopentane

Version 1

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ABSTRACT

Introduction

This protocol describes how to freeze tissues in OCT using dry-ice cooled isopentane (for downstream cryo-sectioning).

Related Documents

Risk assessment: WTSI-3200_ Freezing Tissue using Isopentane

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

Brief outline of major risks

Ergonomic Risks when working in this facility

- The processes undertaken by this facility results in a high risk of Ergonomic stresses.
- Remember to take regular breaks between tasks to minimise these risks, making sure to move and stretch out your limbs. Always sit at the hood with feet on the floor (or foot rest), back straight and arms as close to your sides as possible to retain good posture.
- Maintain general awareness of ergonomic stresses and reduce or avoid these stresses where possible. This could be by using aids such as Multi channel or repeat pipettes wherever possible to reduce repetitive use of hands, fingers and thumbs.

MATERIALS TEXT

Material	Supplier Info
Iso-pentane	ThermoFisher (**10468030**)
2 pairs of Forceps	ThermoFisher UK Ltd (15232290)
Scalpel	Swann-Morton Ltd (0502)
Labelled Cryotubes	
Cryomold Biopsy 10 x 10 x 5mm (Pk100);	Agar Scientific (AGG4581)
Cryomold, disposable 7 x 7 x 5 mm (pk1000);	Simport (M475-1) (from Thermofisher).

OCT embedding media	VWR (Qpath) 00411243

Equipment	Location
Polystyrene box of dry ice	
Metal Container	
-100°C Thermometer	
-80°C Freezer	
Cold resistant gloves	

Method
Prior to commencing work decontaminate the work area.
Unfixed human tissue work should be performed under sterile conditions in a fume hood or ducted microbiological safety cabinet at containment level 2.
Work with tissues from other species (e.g. mouse) should be performed in a fume hood or downflow table.

SAFETY WARNINGS

Chemical risks

- This protocol should be performed under a fume hood or under a ducted MSC.
- Always wear correct PPE (which includes eye protection, nitrile gloves, thermal gloves for handling Liquid Nitrogen / dry-ice cooled iso-pentane and appropriate labcoat) when handling any chemical.
- For more chemical information see the **COSHH** forms or **MSDS** for each chemical.

Any chemicals which have specific risks and handling instructions will be outlined in the appropriate SOP method section

Biological Risks when working with primary tissues / cell lines from humans

- Cells from primary samples may contain uncharacterised adventitious agents, including blood-borne viruses. No attempt will be made to culture these agents deliberately. Correct use of PPE will drastically reduce the risks. As a facility we offer Hepatitis B vaccinations as standard.
- Viruses used in this facility can infect human cells but are non-replicating and therefore the Pathogenicity of these viruses is negligible. Correct use of PPE will drastically reduce the risks.
- All unfixed human tissue work should be performed at containment level

1 On a downflow or fume hood: Place metal container on dry ice and half fill with isopentane.(use enough iso-pentane to completely immerse the tissue pieces).Iso-pentane is extremely flammable and may be fatal if swallowed or enters airways. To be used in a fume-hood or downflow table wearing correct PPE.

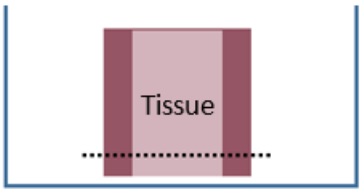
2 Cool 1 pair of forceps in the dry ice. Keep the other pair at room temperature. If used, place labelled cryotube on dry ice.

3 Put lumps of dry ice into the iso-pentane and monitor with the -100°C thermometer until it measures -70°C.

4 Label a cryomold and foil with the sample id

5 Trim the tissue into a piece small enough to fit into the cryomold, if required.

6 Half fill the cryomold with OCT and position the tissue in the cryomold.Note: for spatial transcriptomics use small cryomolds (maximum 10 x10 mm)



Ensure that the tissue is oriented correctly to achieve the desired cryosection. E.g. Place tissue with region of interest to section, pushed flat at the bottom of the mold. Ensure there is a thin layer of OCT at the bottom of the mold so the tissue is fully covered. Dotted line in the image above shows how cross-sections would be cut from the tissue. Sections would be generated from the bottom of the block upwards.

7 Add more OCT if needed to ensure the tissue is fully submerged and covered in OCT.

8 Using forceps, submerge the cryomold in the container of isopentane, chilled to -70°C on dry ice.

9 When completely frozen, wrap sample in foil (still in cryomold), and keep on dry ice. Alternatively, remove sample from cryomold and place in labelled cryotube on dry ice.

10 Transfer samples on dry ice, to labelled box in -80°C freezer.

11 If freezing more samples monitor the isopentane temperature and adjust as necessary.

12 Wearing cold-resistant gloves, carefully remove the metal container of iso-pentane from the dry ice. The iso-pentane and dry ice can be left with the lid off, in a fume hood or downflow table to evaporate – ensure it is clearly labelled.Alternatively, the iso-pentane can allowed to warm to -10C, then decanted to a suitable bottle for re-use. The bottle must be labelled and kept in a fume hood/ downflow table, with the lid loosened until the iso-pentane returns to room temperature.