Freezing of Tissue in Optimal Cutting Temperature

Category:	**Materials Handling and Documentation**		
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1.0 PURPOSE

The purpose of this SOP is to establish standardized procedures for the freezing of tissue samples in Optimal Cutting Temperature (OCT) compound at Nourah's Tissue Biobank. This technique is essential for preserving tissue morphology for future cryosectioning and histological analysis.

2.0 SCOPE

This SOP applies to all personnel involved in the freezing of tissue samples using OCT compound within Nourah's Tissue Biobank. It covers the steps required to properly embed tissue in OCT, freeze the samples, and document the process using the LabVantage LIMS system.

3.0 ROLES AND RESPONSIBILITIES

This SOP applies to all personnel of Nourah's Tissue Biobank members

Biobank Personnel	Responsibility
Pathologist/Pathologist Assistant	Responsible for selecting and preparing tissue samples for OCT embedding, ensuring they meet the necessary criteria.
Laboratory Technicians	Responsible for performing the OCT embedding and freezing procedures according to this SOP, ensuring the proper handling, labeling, and documentation of tissue samples.
Biobank Manager	Responsible for overseeing the OCT freezing process, ensuring compliance with this SOP, and addressing any issues that arise.
Quality Assurance (QA) Officer	Responsible for auditing the OCT freezing process, ensuring adherence to protocols and regulatory requirements.

4.0 MATERIALS, EQUIPMENT, AND FORMS

Listing of the materials, equipment, and forms being used to achieve the goals of the SOP, this list will mainly contain necessary materials and, or recommendations that may be substituted by alternative or equivalent materials more suitable at the time of testing.

Material to be used	Site
OCT compound	
Tissue embedding molds	
Liquid nitrogen or dry ice-isopentane bath	
Isopentane (when using dry ice for freezing)	
Cryovials or labeled containers for frozen samples	
Labels and markers	
Forceps and scalpel	
Insulated gloves and face shield (PPE)	
Insulated transport containers (e.g., dry shippers)	
Biohazard waste containers	

5.0 POTENTIAL HAZARDS

In this part of the SOP, we explain the potential hazards from chemicals and methodologies used in this procedure. It will also contain information on how to handle these chemicals and the level of biosafety

Material	Safety and handling

6.0 PROCEDURES

This section outlines the steps involved in embedding tissue samples in OCT compound, freezing the embedded samples, and documenting the process in Nourah's Tissue Biobank. These procedures ensure that tissue morphology is preserved for accurate cryosectioning and subsequent analysis.

6.1 PRE-FREEZING PREPARATION

1. The pathologist or pathologist assistant selects tissue samples suitable for embedding in OCT, considering the research or diagnostic needs.
2. Ensure that tissue samples are appropriately sized for embedding in molds, typically no thicker than 0.5 cm.
3. Prepare tissue embedding molds by filling them partially with OCT compound.
4. Place the molds on a cold surface or on dry ice to begin cooling the OCT compound before tissue embedding.
5. Wear appropriate PPE, including insulated gloves and a face shield, to protect against the extreme cold during the freezing process.
6. Ensure that the work area is well-ventilated, especially when using isopentane.

6.2 EMBEDDING AND FREEZING TISSUE IN OCT

1. Using sterilized forceps, carefully position the tissue sample in the center of the OCT-filled mold, ensuring that the tissue orientation is correct for future cryosectioning.
2. Cover the tissue completely with additional OCT compound, filling the mold to the top.
    a. **Using Liquid Nitrogen:** Place the OCT-filled mold with the tissue directly into liquid nitrogen until fully frozen (usually within a few minutes).
    b. **Using Isopentane Bath:** Lower the OCT-filled mold into the isopentane bath cooled by liquid nitrogen until the tissue is fully frozen.
3. Ensure that the OCT solidifies rapidly, which is critical for preserving tissue morphology.
4. Once the OCT is fully solidified and the tissue is frozen, remove the embedded tissue block from the mold if needed, and place it in a pre-labeled cryovial or another suitable storage container.

6.3 LABELING AND DOCUMENTATION

1. Ensure all cryovials are labeled with the necessary information, including patient identifier, tissue type, date, and freezing method used.
2. Verify that the labeling is legible and securely attached to the cryovials.
3. Enter the details of the OCT frozen tissue samples into the LabVantage LIMS system immediately after freezing.
4. Document the method of freezing, time, and any observations regarding the condition of the tissue before and after freezing.

6.4 TRANSPORTATION

1. Store the cryovials containing the frozen tissue samples in liquid nitrogen or an ultra-low temperature freezer (-80°C or colder) as soon as possible after freezing.
2. Ensure that the storage location and conditions are updated in the LabVantage LIMS system for accurate tracking.
3. Snap frozen tissue samples need to be transported, place the cryovials in an insulated transport container with liquid nitrogen or dry ice, depending on the destination.
4. Record the transport details in the LIMS, ensuring traceability during transportation.

6.5 INCIDENT MANAGEMENT

1. In case of any deviations from the SOP (e.g., temperature excursions, delays), document the incident and notify the Biobank Manager immediately.
2. Implement corrective actions as necessary to mitigate any impact on sample integrity.
3. Complete an incident report detailing the deviation, corrective actions taken, and any follow-up measures required.
4. Submit the report to the QA Officer for review and documentation.

7.0 REFERENCES

1. CTRnet SOPs "08.03.004 Freezing of Tissue OCT"
2. Declaration of Helsinki.
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3. Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics
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4. Best Practices for Repositories I. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER).
   http://www.isber.org/Search/search.asp?zoom_query=best+practices+for+repositories
5. National Bioethics Advisory Commission: Research involving human biological materials: Ethical issues and policy guidance, Vol. I: Report and recommendations of the National Bioethics Advisory Committee. August 1999.
   http://bioethics.georgetown.edu/nbac/hbm.pdf
6. US National Biospecimen Network Blueprint
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8.0 REVISION HISTORY

SOP No.	Date Revised	Author	Summary

9.0 APPENDICES