Title	Freezing Tissue for Biorepositories
SOP Code	SOP115_02
Effective Date	04-Jan-2016

Site Approvals

Name and Title (typed or printed)	Signature	Date dd/Monyyy

1.0 PURPOSE

This Standard Operating Procedure (SOP) outlines standardized procedures for biorepositories to snap freeze tumour tissue. This SOP does not describe detailed safety procedures for handling human biological materials (HBMs), or hazardous chemicals.

2.0 SCOPE

This procedure ensures that tissue specimens collected from participants will be frozen in a safe and efficient manner, while eliminating the risks of contamination, and loss of molecular integrity.

3.0 RESPONSIBILITIES

This procedure applies to all biorepository personnel responsible for freezing harvested tissue. Freezing is performed by the laboratory technician, or trained personnel designated by the tumour repository.

4.0 DEFINITIONS

See Glossary of Terms.

5.0 PROCEDURE

5.1 Snap Freezing of Tumour Tissue

5.1.1 Have materials and equipment ready. Have the required number of cryovials labelled and ready.

5.1.2 Freeze fresh tumour tissue as soon as possible, unless intended for another method of preservation. Optimally, freeze tissue within 30 minutes from resection.

5.1.3 Do not freeze the tissue directly on ice.

5.1.4 Ensure that the resected tissue never desiccates, or is contaminated by surrounding tissue or other samples. Use clean scalpels and forceps between samples to avoid cross contamination between samples or between tumour and normal tissue.

5.1.5 Snap frozen tissue is suitable for preparation of DNA, RNA and protein. Do not place the sample in contact with formalin at any point in the process. Do not add serum to the sample.

5.1.6 Cool isopentane by suspending the container of isopentane in liquid nitrogen. Isopentane is sufficiently cooled when “pearls” form, and the solution becomes hazy.

5.1.7 Using clean forceps, place the specimen to be frozen into an empty screw capped cryovial.

5.1.8 Close the cryovial.

5.1.9 Place the cryovial with the specimen into the container of cooled isopentane. The specimen should freeze within 30 seconds.

5.1.10 Alternatively, the isopentane freezing step can be the optional. Place the tissue specimen into an empty cryovial, close the cryovial, and immediately submerge the cryovial into liquid nitrogen. The specimen should freeze within 30-60 seconds. This is not recommended if the sample is large in size, as longer freezing time will result in ruined morphology.

5.1.11 Transfer the sample to liquid nitrogen storage container (preferred), or to a -80º C (or colder) freezer after it is snap frozen.

5.1.12 Place samples on dry ice to be carried to the freezer, or liquid nitrogen storage facility.

5.1.13 Place samples in the vapour phase, if storing in liquid nitrogen, < 150 º C.

5.1.14 Record the storage location, and time of freezing.

5.2 Freezing of Tissue in Optimal Cutting Temperature (OCT) Medium

5.2.1 Have materials and equipment ready. Have as many cryovials or cryomolds as needed labelled and ready.

5.2.2 Freeze fresh tumour tissue as soon as possible, unless intended for another method of preservation. Optimally, freeze tissue within 30 minutes from resection.

5.2.3 Do not freeze the tissue directly on ice.

5.2.4 Ensure that the resected tissue never desiccates, or is contaminated by surrounding tissue or other samples. Use clean scalpels, and forceps between samples to avoid cross contamination between samples, or between tumour and normal tissue.

5.2.5 OCT frozen tissue is suitable for preparation of DNA and RNA. It is especially useful for preserving fresh tissue intended for histopathology where morphological information is important. Do not place the sample in contact with formalin at any point in the process. Do not add serum to the sample.

5.2.6 Cool isopentane by suspending the container of isopentane in liquid nitrogen. Isopentane is sufficiently cooled when “pearls” form and the solution becomes hazy.

5.2.7 Place a few drops of the OCT compound into a pre-labelled plastic cryomold.

5.2.8 Using clean forceps, place the specimen to be frozen onto the OCT in the cryomold. If relevant to the type of tumour specimen, orient the tissue in the cryomold.

5.2.9 Add more OCT to cover the tissue, and fill the mold.

5.2.10 Use forceps or a transfer pipette to orient the tissue, and remove air bubbles.

5.2.11 Avoid introducing any air bubbles into the OCT. Release any bubbles that may become trapped around the tissue.

5.2.12 Place the crymold in a small container containing pre-cooled isopentane.

5.2.13 Submerge the mould in the isopentane until the OCT is completely frozen (white and solid).

5.2.14 Alternatively, the cryomold or cryovial containing the tissue, and OCT can be frozen directly in liquid nitrogen without the isopentane step. Hold the vial or cryomold with forceps, and gently immerse the mould in liquid nitrogen. Liquid nitrogen should be contained in a dry shipper, allowing for freezing to proceed from the bottom of the cryomold or vial.

5.2.15 Remove the mold from the liquid nitrogen.

5.2.16 Place the mold into a small labelled zip-lock bag, and place the sealed bag on dry ice.

5.2.17 Transport the bags or vials on dry ice, and for storage at -80º C or colder.

5.2.18 Record the storage location, and time of freezing.

6.0 REFERENCES

Health Canada, Food and Drug Regulations, Part C, Division 5, Drugs for Clinical Trials Involving Human Subjects, (Schedule 1024), June 20, 2001.

Health Canada, Guidance for Industry, Good Clinical Practice: Consolidated Guideline, ICH Topic E6, 1997.

2011 NCI Best Practices for Specimen Resources. Office of Biorepositories and Biospecimen Research, National Cancer Institute, Bethesda, MD. http://biospecimens.cancer.gov/bestpractices/2011-NCIBestPractices.pdf

ISBER Best Practices for repositories: Collection, storage, retrieval and distribution of biological materials for research. 3rd Edition, 2012. http://www.isber.org

CTRNET Standard Operating Procedures, Canadian Tissue Repository Network

7.0 REVISION HISTORY

SOP Code	Effective Date	Summary of Changes
SOP115_01	01-Aug-2012	Original version
SOP115_02	04-Jan-2016	1.0 Purpose, 5.1.1, 5.1.7, 5.2.8, 5.2.10, 5.14: Clarification added. Updated references. Removed OTRN logo.