Title	Nucleic Acid Extraction from Tissue
SOP Code	SOP119_01
Effective Date	01-Sep-2012

Site Approvals

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

1.0 PURPOSE

This Standard Operating Procedure (SOP) outlines standardized procedures for biorepositories to follow when extracting nucleic acids from tissue specimens. This SOP does not describe detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals.

2.0 SCOPE

This is the suggested process for RNA extraction from snap frozen tissue and tissue frozen in Optimal Cutting Temperature (OCT) medium and DNA extraction from snap frozen tissue.

3.0 RESPONSIBILITIES

This procedure applies to all biorepository personnel responsible for extracting RNA or DNA from tissue.

4.0 DEFINITIONS

See Glossary of Terms.

5.0 PROCEDURE

5.1 General Extraction Considerations

5.1.1 Due to the sensitivity of nucleic acid amplification technologies, precautions should be taken to avoid cross contamination of samples.

5.1.2 Avoid moistening the rim of the spin columns with pipette tips and avoid touching the column with the pipette tip.

5.1.3 Always use aerosol-barrier tips.

5.1.4 Avoid cross-contamination after each vortexing step, briefly centrifuge the tubes to remove droplets that may be on the lids of the tubes.

5.1.5 Close the lids of the spin columns before placing in the microcentifuge.

5.1.6 Flow-through generated after each centrifugation step may contain hazardous materials. Dispose of appropriately.

5.1.7 Open only one spin column at a time, and avoid creating aerosols.

5.1.8 Do not use any plastic-ware or glassware without first eliminating RNase or DNase contamination.

5.1.9 Take care not to introduce RNase or DNase into the sample during or after the purification procedure.

5.1.10 It is optimal to use sterile RNase-free or DNase-free disposable vessels and solutions while working with nucleic acids. Microbiological aseptic technique is always optimal to use when working with nucleic acids.

5.1.11 Wear latex or vinyl gloved while handling reagents, tubes, and samples to prevent RNase and DNase contamination from the skin or surface of the laboratory. Change gloves frequently.

5.1.12 Keeps tubes closed, whenever possible.

5.1.13 Keep purified RNA on ice.

5.1.14 Keep samples frozen below -80º C or lower for long term storage.

5.2 Extraction of RNA from Frozen Tissue

Note: Volumes indicated are recommendations only, and should be scaled according to the size of the tissue specimen. A commercial RNA extraction kit is suggested for RNA extraction from specimens are small in size.

5.2.1 Have materials and equipment ready before starting the procedure. Have as many tubes and cryovials as needed labelled and ready.

5.2.2 Homogenization: Keep tissues frozen at -80ºC until homogenization.

5.2.3 Homogenize tissue samples in 1 ml of TRIZOL reagent per 50-100 mg of tissue using a glass-Teflon or power homogenizer. Alternate RNase free methods for homogenizing frozen tissue can be used if a homogenizer is not available.

5.2.4 The sample volume should not exceed 10% of the volume of the TRIZOL used for homogenization.

5.2.5 Phase Separation: Incubate the homogenized samples for 5 minutes at room temperature, to permit complete dissociation of nucleoprotein complexes.

5.2.6 Add 0.2 ml of chloroform per 1 ml of TRIZOL reagent. Cap tubes securely and shake tubes vigorously by hand for 15 seconds.

5.2.7 Incubate at room temperature for 2-3 minutes.

5.2.8 Centrifuge the sample at no more than 12,000 x g for 10 minutes, in a refrigerated centrifuge (2-8ºC).

5.2.9 Following centrifugation, the mixture separates into distinct phases: a lower red phenol/chloroform phase, an interphase and a colourless upper aqueous phase.

5.2.10 RNA remains exclusively in the aqueous phase and this phase is about 60% of the volume of the TRIZOL reagent used for homogenization.

5.2.11 RNA precipitation: Transfer the aqueous phase to a clean tube (the organic phase may be saved if isolation of DNA or protein is required from this same sample).

5.2.12 Precipitate the RNA from the aqueous phase by mixing with isopropyl alcohol. Use 0.5 ml of isopropyl alcohol per 1ml of TRIZOL reagent used for the original homogenization.

5.2.13 Incubate at room temperature for 10 minutes.

5.2.14 Centrifuge the sample at 180000 x g for 10 minutes in a refrigerated centrifuge (2-8ºC).

5.2.15 The precipitated RNA forms a gel-like pellet on lower side and bottom of the centrifuge tube.

5.2.16 Washing steps: Remove the supernatant.

5.2.17 Wash the RNA pellet once with 75% ethanol adding at least 1 ml of 75% ethanol per 1 ml of TRIZOL reagent initially used for homogenization.

5.2.18 Mix the sample by gentle vortexing.

5.2.19 Centrifuge at 7500 x g for 5 minutes, in a refrigerated centrifuge (2-8ºC).

5.2.20 Redissolving the precipitated RNA: Briefly dry the RNA pellet. Air dry or vacuum dry for 5-10 minutes. Take care not to completely dry the pellet as this will cause difficulty with dissolving the RNA.

5.2.21 Dissolve the pellet in an appropriate volume of RNase free water.

5.2.22 Store the dissolved RNA at -80ºC or lower.

5.2.23 Record the storage location.

5.3 Extraction of RNA from Tissue Frozen in OCT

Note: Volumes indicated are recommendations only and should be scaled according to the size of the tissue sample. A commercial RNA extraction kit is suggested for RNA extraction from OCT sections.

5.3.1 Have materials and equipment ready before starting the procedure. Have as many tubes and cryovials as needed labelled and ready.

5.3.2 Take several (5-10) 3µm OCT sections using a cryostat and place them in a pre-cooled microfuge tube. Ensure that the sections do not thaw before the next step.

5.3.3 Follow the detailed procedure outlined in the RNA extraction kit manual.

5.3.4 Immediately after the procedure, place extracted and resuspended RNA on ice.

5.3.5 Store extracted RNA at -80ºC or lower.

5.3.6 Record the storage location.

5.4 Extraction of DNA from Frozen Tissue

5.4.1 Have materials and equipment ready before starting the procedure. Have as many tubes and cryovials as needed labelled and ready.

5.4.2 Cut tissue (mince) into small pieces with a sterile scissors or scalpel blade. Alternatively, wrap frozen tissue in aluminum foil and fragment with a hammer.

5.4.3 Follow the detailed procedure outlined in the DNA extraction kit manual.

5.4.4 Store DNA at 4ºC, or at -20º C or lower for longer term storage.

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. Cell Preservation Technology 6(1), 3-58, 2008 http://www.isber.org/Pubs/BestPractices2008.pdf

CTRNET Standard Operating Procedures, Canadian Tumour Repository Network, http://www.ctrnet.ca/operating-procedures

7.0 REVISION HISTORY

SOP Code	Effective Date	Summary of Changes
SOP119_01	01-Sep-2012	Original version