CTRNet Standard Operating Procedure
Tisssue Derivatives: Extraction of RNA

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REVISION HISTORY

SOP Number	Date Issued	Author (Initials)	Summary of Revisions
LP 002.001	2005	JdSH	CTRNet Generic SOP for Collection and Processing of Tumour Tissue
8.3.009	2008	JdSH	Revised to cover extraction of RNA only

1.0 PURPOSE

Tissue samples are collected from patients that have been through the informed consent process and agreed to participate in the tumour repository program. Genomic studies often utilize nucleic acids (DNA and RNA) derived from these samples. When extracting and storing RNA from tissue specimens all efforts should be made to avoid contamination, prevent degradation and preserve molecular integrity. The purpose of this document is to outline standardized procedures for CTRNet repositories to follow when extracting RNA from tissue samples.

2.0 SCOPE

The Standard Operating Procedure (SOP) describes how RNA should be extracted from snap frozen tissue and tissue frozen in OCT. The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals and it is recommended that personnel follow institutional safety guidelines.

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3.0 REFERENCE TO OTHER POLICIES AND SOPS

1. CTRNet Policy: POL 005.001 Records and Documentation
2. CTRNet Policy: POL 002.001 Ethics
3. CTRNet Policy: POL 004.001 Privacy and Security
4. CTRNet Policy: POL 007.001 Material and Information Handling Policy
5. CTRNet Generic Procedure: FS 002.001 CTRNet Generic SOP for Collection and Processing of Tumour Tissue
6. CTRNet SOP: 8.3.003 Preservation of tissue: Snap Freezing
7. CTRNet SOP: 8.3.004 Preservation of tissue: Freezing in OCT
8. CTRNet SOP: 8.3.006 Sectioning of paraffin and OCT embedded tissue
9. CTRNet SOP: 8.1.002 Biohazardous Material Waste Management

4.0 ROLES AND RESPONSIBILITY

The policy applies to all personnel from CTRNet member repositories who are responsible for extracting RNA from tissue.

Tumour Bank Personnel	Responsibility/Role	Site Specific Personnel and Contact Information
Lab Technician	Responsible for labelling tubes and extracting RNA from tissue and storing samples and documenting storage.	

5.0 MATERIALS, REAGENTS, EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	Materials and Equipment (Site-Specific)
Markers, ink and pens	
Appropriate labels for vials and microfuge tubes	
Microfuge tubes	
Racks for Microfuge tubes	
Tissue Homogenizer such as Polytron	
OR Tissue Homogenizer such as glass-TEFLON homogenizer	
Refrigerated Microcentrifuge	
Cryotome	

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Micropipettors	
Sterile Rnase free pipette tips	
TRIZOL® Reagent	
RNeasy Micro Kit (Qiagen)	
Rnase free water	
Isopropyl Alcohol	
Chloroform	
75% Ethanol	
-80º C or -20º C freezer	
Ice for cooling TRIZOL and tubes and water	
Dry ice for transporting OCT blocks or frozen tissue	

*Consult Appendix 1 for the preparation of RNA Extraction Solutions and Buffers. For additional details on preparing buffers see reference #10 (Section 8)

6.0 DEFINITIONS

Cryotome: A device that consists of a Microtome placed inside a freezer and used for sectioning frozen tissue.

DNA: Deoxyribonucleic acid

Microtome: Device used to cut sections from a block to place on slides.

OCT: "Optimal Cutting Temperature" compound is the name used for polyethylene glycol/sucrose-based freezing medium. OCT preserves ultrastructure and prevents tissue from desiccation, degradation, acts as an insulator from thermal variation and minimizes crystal formation. It is especially useful for preserving fresh frozen that may need to be sectioned.

Preservation: Use of chemical agents, alterations in environmental conditions or other means during processing to prevent or retard biological or physical deterioration of a specimen.

RNA: Ribonucleic acid

7.0 PROCEDURES

This procedure is intended to ensure that RNA is extracted from tissue samples in a safe and consistent manner while eliminating the risks of contamination and loss of molecular and structural integrity. Consistency in procedure is important for obtaining comparable and reliable test results. The following steps are based on procedures followed at The Cancer Network of the Fonds de la recherche en santé du Québec (FRSQ).

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7.1 Extraction of RNA from frozen tissue

NOTE: Volumes indicated are recommendations only and should be scaled according to the size of the tissue sample. Make sure that all tubes, homogenizers etc. used in the RNA extraction process are RNase free or treated with RNase inhibitors**.**

1. Treat all tissue as potentially infectious.
2. RNA Extraction is performed by the laboratory technician or trained personnel designated by the tumour repository.
3. Have materials and equipment ready. Have as many tubes and cryovials as needed labelled and ready. All equipment and reagents that come in contact with the sample should be Rnase free.
4. **Homogenization.** Tissue are kept frozen at -80º C until homogenization.
5. 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.
6. The sample volume should not exceed 10% of the volume of the TRIZOL used for homogenization.
7. **Phase Separation.** Incubate the homogenized samples for 5 minutes at room temperature to permit complete dissociation of nucleoprotein complexes.
8. Add 0.2 ml of chloroform per 1 ml of TRIZOL reagent. Cap tubes securely and shake tubes vigorously by hand for 15 seconds.
9. Incubate at room temperature for 2-3 minutes.
10. Centrifuge the sample at no more than 12,000 x g for 10 minutes in a refrigerated centrifuge (2-8º C).
11. Following centrifugation, the mixture separates into distinct phases: a lower red phenol/chloroform phase, an interphase and a colourless upper aqueous phase.
12. RNA remains exclusively in the aqueous phase and this phase is about 60% of the volume of the TRIZOL reagent used for homogenization.
13. **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).
14. Precipitate the RNA form 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.
15. Incubate at room temperature for 10 minutes.
16. Centrifuge the sample at 180000 x g for 10 minutes in a refrigerated centrifuge (2-8º C).

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17. The precipitated RNA forms a gel-like pellet on lower side and bottom of the centrifuge tube.
18. Washing steps. Remove the supernatant.
19. 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.
20. Mix the sample by gentle vortexing.
21. Centrifuge at 7,500 x g for 5 minutes in a refrigerated centrifuge (2-8º C).
22. **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.
23. Dissolve the pellet in an appropriate volume of RNase free water.
24. Store the dissolved DNA at -80º C or lower.
25. Record the storage location.

7.2 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. The RNeasy Kit is the method of choice for the extraction of RNA especially when the samples are small in size or in the case of RNA extraction from OCT sections.

1. Treat all tissue as potentially infectious.
2. RNA Extraction from tissue embedded in OCT is performed by the laboratory technician or trained personnel designated by the tumour repository.
3. Have materials and equipment ready. Have as many tubes and cryovials as needed labelled and ready. All equipment and reagents that come in contact with the sample should be RNase free.
4. Use Qiagen's RNeasy Mirco extraction kit for RNA isolation from tissue embedded in OCT.
5. Take several (5-10) 3µm OCT sections using a cryostat and place them in a pre-cooled microfuge tube. Make sure that the sections do not thaw before the next step.
6. Add 600µl RLT Buffer* and bring to room temperature.
7. Centrifuge for 12 minutes at maximum speed (>180000 x g).
8. Remove supernatant fluid, but not surface layer, into new tube. Discard the rest.
9. Add 600µl 70% ethanol, mix using pipet.

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10. Take up to 700µl of that solution and run through a supplied mini column, centrifuge for 15 seconds at 8000 x g.
11. Repeat the previous step until all of the solution has run through the mini column.
12. Run 700µl RW1 Buffer* through the mini column, centrifuge 15 seconds at 8000 x g.
13. Change the collection tube under the mini column.
14. Run 500µl RPE Buffer* through the column, centrifuge 15 seconds at 8000 x g.
15. Centrifuge another 500µl RPE Buffer * through the column, but this time for 2 minutes at maximum speed.
16. Change out the collection tube, spin to ensure that the column is dry for 1+ minute(s) at maximum speed. If any fluid collects in the tube, spin for another minute or two.
17. Add 30µl RNase-free H2O directly to the filter of the column, let incubate for 5-10 minutes, and spin for 1 minute at 8000 x g.
18. Store extracted RNA as above.

*The reagents (RLT, RW1, and RPE) are all supplied in the RNeasy kits (see Ref. 12 for details)

8.0 APPLICABLE REFERENCE, REGULATONS AND GUIDELINES

1. Declaration of Helsinki. http://ohsr.od.nih.gov/helsinki.php3 http://www.wma.net/e/policy/b3.htm
2. Tri-Council Policy Statement; Ethical Conduct for Research Involving Humans; Medical Research Council of Canada; Natural Sciences and Engineering Council of Canada; Social Sciences and Humanities Research Council of Canada, August 1998. http://www.pre.ethics.gc.ca/english/policystatement/policystatement.cfm
3. Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics Series. http://www.mrc.ac.uk/pdf-tissue_guide_fin.pdf
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
5. US National Biospecimen Network Blueprint http://www.ndoc.org/about_ndc/reports/NBN_comment.asp
6. Jewell, S. et al. 2002, Analysis of the Molecular Quality of Human Tissues, an experience from the Cooperative Human Tissue Network. Am. J. Clin. Pathol. 118:733-741.

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7. Guideline – Fresh Tissue Working Group of BIG and NCI breast cancer Cooperative Groups http://ctep.cancer.gov/forms/guidelines_fresh_tissue.pdf

8. Snell L. and P. H. Watson. 2006, Breast Tissue Banking: Collection, Handling, Storage and Release of Tissue for Breast Cancer Research. Methods Mol Med. 120:3-24.

9. RNA Extraction procedure from Fonds de la recherche en santé du Québec (FRSQ).

10. Procedure for the RNA Extraction from tissues in OCT - from Fonds de la recherche en santé du Québec (FRSQ).

11. Product Sheet for TRIZOL® Reagent. Form No. 18057N. INVITROGEN Life Technologies.

12. RNeasy Micro Handbook http://www1.qiagen.com/literature/handbooks/PDF/RNAStabilizationAndPurification/FromAnimalAndPlantTissuesBacteriaYeastAndFungi/RNY_Micro/1023761_HBRNY_Micro0403.pdf

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