CTRNet Standard Operating Procedure Tissue Derivatives – Extraction of RNA			
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Approved By:	CTRNet Management Group (CMG)	01-June-2012
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1.0 PURPOSE

Tissue samples are collected from patients that have been through the informed consent process and agreed to participate in the tumour biobank 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.

2.0 SCOPE

The standard operating procedure (SOP) describes how RNA should be extracted from snap frozen tissue and tissue frozen in Optimal Cutting Temperature (OCT) compound. 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.

3.0 REFERENCE TO OTHER CTRNET SOPS OR POLICIES

Note: When adopting this SOP for local use please reference CTRNet.

3.1 *CTRNet Policy: POL 005.001 Records and Documentation*
3.2 *CTRNet Policy: POL 002.001 Ethics*
3.3 *CTRNet Policy: POL 004.001 Privacy and Security*
3.4 *CTRNet Policy: POL 007.001 Material and Information Handling*
3.5 *CTRNet Standard Operating Procedure: SOP 08.03.003 Snap Freezing of Tissue*
3.6 *CTRNet Standard Operating Procedure: SOP 08.03.004 Freezing of Tissue in OCT*
3.7 CTRNet Standard Operating Procedures: *SOP 08.03.006 Sectioning of Tissue – Paraffin and OCT embedded tissue*
3.8 *CTRNet Standard Operating Procedure: SOP 08.01.002 Biohazardous Material Waste Management*

4.0 ROLES AND RESPONSIBILITIES

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

Tumour Biobank Personnel	**Responsibility/Role**
Laboratory Technician/Technologist	Responsible for labeling tubes and extracting RNA from tissue, storing samples and documenting storage.

5.0 MATERIALS, 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 and microcentrifuge	
Homogenizer such as a TissueRuptor glass-TEFLON homogenizer	
Qiacube automatic extractor	
Qiacube special microtubes,tips and rotor adapters	
Cryotome	
Micropipettors	
Sterile, Rnase-free pipet tips	
RNeasy Mini Kit	
RNeasy Micro Kit	
14.3M ß -Mercaptoethanol (ß-Me) or 2 M dithiothreitol (DTT)	
96-100% ethanol	
Disposable gloves	
70% ethanol	
-80º C or -20º C freezer	
Ice for cooling tubes and water	
Dry ice for transporting OCT blocks or frozen tissues	

*Consult Appendix A for the preparation of RNA Extraction Solutions and Buffers. For additional details on preparing buffers see reference 8.10.

6.0 DEFINITIONS

See the CTRNet Program Glossary: http://www.ctrnet.ca/glossary

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 extractions protocols for quality control conducted by CTRNet.

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**.** This protocol relies on the RNeasy Mini kit.

7.1.1 Treat all tissue as potentially infectious.

7.1.2 RNA extraction is performed by the laboratory technician/technologist or trained personnel designated by the tumour biobank.

7.1.3 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.

7.1.4 Homogenization. Tissue samples are kept frozen at -80º C until homogenization.

7.1.5 Homogenize tissue samples (maximum 30 mg) in 600 uL of Buffer RLT* using a glass-Teflon or power homogenizer. Alternate RNase free methods for homogenizing frozen tissue can be used if a homogenizer is not available. (Refer to Step 3 of RNeasy Mini Handbook in the section entitled Animal Tissues).

7.1.6 Centrifuge the lysate for 3 min at full speed (>18,000 x g). Carefully remove the supernatant by pipetting, and transfer it to a new microcentrifuge tube.

7.1.7 Proceed with the Rneasy Mini Qiacube protocol, or for manual processing continue with steps 5-12 described in the RNeasy Mini Handbook.

7.1.8 Store the dissolved RNA at -80º C or lower.

7.1.9 Record the storage location.

7.2 Extraction of RNA from Tissue Frozen in OCT

NOTE: This protocol relies on the RNeasy Micro kit, which is favoured, as tissue sections tend to be small. However, for larger samples, use the RNeasy Mini kit as described above.

7.2.1 Treat all tissue as potentially infectious.

7.2.2 RNA extraction from tissue embedded in OCT is performed by the laboratory technician/technologist or trained personnel designated by the tumour biobank.

7.2.3 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.

7.2.4 Use RNeasy Micro extraction kit for RNA isolation from tissue embedded in OCT.

7.2.5 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.

7.2.6 Add 600µl Buffer RLT * and bring to room temperature.

7.2.7 Centrifuge for 12 minutes at maximum speed (>18,0000 x g).

7.2.8 Remove supernatant fluid, but not surface layer, into new tube. Discard the rest.

7.2.9 Add 600µl 70% ethanol, mix using a pipet.

7.2.10 Take up to 700µl of that solution and run through a supplied mini column. Centrifuge for 15 seconds at 8,000 x g.

7.2.11 Repeat the previous step until all of the solution has run through the mini column.

7.2.12 Run 700µl Buffer RW1* through the mini column, centrifuge 15 seconds at 8,000 x g.

7.2.13 Change the collection tube under the mini column.

7.2.14 Run 500µl Buffer RPE* through the column, centrifuge 15 seconds at 8,000 x g.

7.2.15 Centrifuge another 500µl Buffer RPE * through the column, but this time for 2 minutes at maximum speed.

7.2.16 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.

7.2.17 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.

7.2.18 Store extracted RNA as above.

*The reagents (RLT, RW1, and RPE) are all supplied in the RNeasy kits (see Ref. 8.11, 8.12, and 8.13 for details).

8.0 APPLICABLE REFERENCES, REGULATIONS AND GUIDELINES

8.1 Declaration of Helsinki
http://www.wma.net/en/30publications/10policies/b3/index.html

8.2 Tri-Council Policy Statement 2; 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, December 2010.
http://www.pre.ethics.gc.ca/eng/policy-politique/initiatives/tcps2-eptc2/Default/

8.3 Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics
http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002420

8.4 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

8.5 US National Biospecimen Network Blueprint
http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp

8.6 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.

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

8.8 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.

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

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

8.11 Rneasy Mini Handbook
http://www.qiagen.com/literature/render.aspx?id=352

8.12 Rneasy Mini Qiacube Handbook
http://www.qiagen.com/literature/render.aspx?id=200915

8.13 RNeasy Micro Handbook – http://www.qiagen.com

9.0 APPENDICES

None

10.0 REVISION HISTORY

SOP Number	Date revised	Author	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
8.3.009 e1.0	May 2012	CMG	• Grammatical and formatting throughout • Definitions removed • Revision History moved to bottom • Reference links updates • Updated SOP references • Section 1.0 – Deleted “the purpose of this…” • Section 5.0 – Table updated • Section 7.0 – Procedures modified