STANDARD OPERATING PROCEDURE	**SOP No. 006** **RNA Extraction from Frozen Tissue** **UW Network Site**
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1. PURPOSE

To describe the procedure for the extraction of RNA from frozen tissue. NOTE: This SOP does not cover detailed procedures for handling Human Biological Materials or hazardous chemicals and it is recommended that personnel following this SOP refer to institutional safety guidelines.

The following SOP for RNA extraction from frozen tissue is followed by the UW Network Site of the PCBN.

2. RESPONSIBILITIES

Authorized personnel extracting RNA from frozen prostate tissue must ensure that:

- all procedures are followed correctly
- all samples are adequately coded during processing
- all documentation is completed, and accurate records maintained on all samples

3. HEALTH AND SAFETY

Personnel carrying out this procedure must maintain safe working practices and observe all relevant Environmental Health and Safety (EH&S) guidelines. This includes the appropriate use of Personal Protective Equipment (PPE), and the procedures for waste disposal, disinfection & spill clean-up and biosafety.

4. EQUIPMENT AND MATERIALS

Equipment	Materials
Centrifuge Cryostat Fume hood gentleMACS Dissociator Refrigerated Microcentrifuge Vortex	Chloroform Cryovials 75% ethanol isopropyl alcohol gentleMACS M tube Microcentrifuge tubes Phase Lock Gel Heavy 2ml tube (5 Prime) RNA STAT-60 RNase-free water Scalpel blades

5. PROCEDURES

5.1 Sample Preparation

- Keep previously frozen tissue tubes on dry ice and frozen until in RNA-Stat 60

If starting with frozen bit of tissue:

- Add 1.1 ml RNA STAT-60 to gentleMACS M tube
- If tissue is less than 100 mg, place directly into M tube, if tissue is more than 100 mg, remove from tube and cut piece to less than 100 mg using a new scalpel blade before placing in M tube.
- After replacing lid, ensure that all of the sample material reaches the area of the roto/stator.
- Run the gentleMACS Program RNA_02 (this is for frozen tissue).
- Centrifuge the M Tube at 2000 RCF for 1 minute at room temperature to collect lysate at the bottom of the tube.
- Remove homogenized sample from the tube and place into a 1.5 ml tube and let incubate at room temperature for 5 minutes.

If starting with 1.5 ml tube of frozen tissue block sections (these should be ≤20 um thick and weigh less than 100 mg):

- Add 1.1 ml of RNA STAT-60 to tube of tissue sections.
- Vortex for 1 minute to lyse tissue sections.
- Incubate for 5 minutes at room temperature.

5.2 RNA Extraction

- Pre-spin Phase Lock Gel Heavy 2ml tube (5 Prime) at 12,000 RCF for 1 minute at 4°C and set aside
- Add 100 ul of nuclease-free water to above pre-spun tube
- Centrifuge samples at 12,000 RRCF for 3 minutes at 4°C to pellet cellular debris and allow possible lipid content to layer on top of sample. If lipid content is observed at the surface of the STAT-60 samples, then pipet to remove.
- Remove 1 ml of STAT-60 sample supernatant by pipetting and place in the previously pre-spun Phase Lock Gel tube. Any remaining RNA STAT-60 can be discarded.
- Add 200 ul of chloroform.
- Cap sample tubes securely. Shake tubes vigorously by hand for 15 secs and incubate at room temperature for 2-3 min.
- Centrifuge the samples at no more than 12,000 RCF for 15 min at 4°C.
- Obtain new 1.5 ml tube, label and add 600ul of isopropanol.
- Transfer the upper aqueous phase to the new 1.5 ml microcentrifuge tube containing isopropanol (this can be easily completed by pouring as the Phase Lock Gel forms a tight seal between the phases).
- Vortex 15 seconds. Allow RNA to precipitate 5 to 7 minutes at room temperature.
- Centrifuge at 12,000 RCF for 10 minutes at 4°C to pellet RNA.

- Remove supernatant and discard.
- Add 1 ml of cold 75% ethanol, invert tube several times to wash RNA pellet.
- Centrifuge at 7,500 RCF for 5 minutes at 4°C.
- Remove supernatant and air dry pellet at room temperature for 5-10 minutes.
- Solubilize RNA in an appropriate amount of cold nuclease-free water.