Molecular Medicine Ireland

SOP 3.11.4 RNA Extraction from Tissue

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Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
Version Number	

Purpose
This SOP describes the procedure for RNA extraction from tissue.

Responsibility
It is the responsibility of the research laboratory personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

General considerations
- RNA can be extracted from formalin fixed and wax embedded tissue but the products are shorter and fragmented, so it is preferable to use the fresh tissue or stored frozen.
- RNAse inhibitors can be used to protect RNA from degradation both during isolation and purification and also in downstream applications such as reverse transcription into cDNA by RT-PCR.

Equipment/reagent requirements
- TRI reagent
- Chloroform
- Isopropanol
- Ethanol
- Homogeniser
- An incubator/water-bath capable of 55-60°C
- RNAse free water
- A refrigerated centrifuge capable of 12,000g
- A vortex mixer
- -80°C Freezer
- A spectrophotometer capable of reading 260 and 280nm/Nanodrop
- BioRad Experion / Agilent Bioanalyser 2100

Procedure
1. Homogenisation: The tissue should be homogenised in 1mL TRI reagent per 50-100mg tissue.

2. Resuspend the cells in 20mL 1X TRI reagent. Store the homogenate for 5 min at RT (18-22°C).
3. RNA extraction: Add 0.1mL bromochloropropane or 0.2mL of chloroform to the mixture and mix vigorously. Store sample for 2-15 min at RT (18-22°C). Centrifuge at 12,000g for 15 min at 4°C.

4. RNA precipitation: Transfer aqueous phase into a new tube. Add 0.5mL of isopropanol and mix, then store for 5-10 min at RT. Centrifuge at 12,000g for 8 min at 4-25°C.

5. RNA wash: Mix RNA pellet with 1mL of 75% ethanol. Centrifuge at 7,500g for 5 min at 4-25°C.

6. Solubilization: Air dry the RNA pellet for 5-10 min. Dissolve by pipetting in 50-200µL of FORMAzol, 0.5%SDS, or DEPC treated RNAase free water and incubate at 55-60°C for 10min.

7. The absorbance of the RNA at 260nm and 280nm should be measured using quartz cuvets or the nanodrop method to assess purity. The 260/280 ratio should be >1.8. An A_{260} of 1.0 in a 1cm light path is equivalent to a RNA concentration of 40µg/mL. The RNA sample is aliquoted and stored at -80°C.

8. An aliquot of the RNA from a representative sample from each batch may be analyzed by electrophoresis through a 0.3% agarose gel in denaturing conditions. Briefly the prepared RNA should be treated with RNA loading buffer 1:2 at 65°C for 10min and loaded into the wells for electrophoresis. The presence of two strongly staining bands, 28S and 18S ribosomal RNAs indicate intact RNA. Degradation is observed by a smear running down the length of the gel.

9. Total and/or mRNA quality and quantity can also be assessed using automated electrophoresis systems such as the BioRad Experion or Agilent Bioanalyser 2100. Determination of RIN may also be used as a measure of quality.

Note: There are a number of commercially available kits based for RNA extraction from different manufacturers. When using these methods follow the manufacturer's

instructions as outlined in the information for use/ package insert included with the kit. The method used for RNA extraction should be recorded in the study specific documentation or data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.