Dublin Academic Medical Centre

Standard Operating Procedure
Dublin Academic Medical Centre
UCD Clinical Research Centre

SOP Number	**5.2**
Version Number	**1**
SOP Title	**RNA Extraction from Blood**

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Purpose

To outline the laboratory procedure for extracting RNA from blood.

Specific procedure

1. Know the location of the Material Safety Data Sheets (MSDS) for all hazardous chemicals used in this procedure (chloroform, 2-mercaptoethanol, CTAB, isopropanol and isoamyl alcohol). Read and become familiar with the safe use of these chemicals. If you have a question or concern regarding health or safety with respect to a specific chemical, consult the laboratory supervisor, or the principal investigator before proceeding.

2. Always use protective clothing when performing this procedure. This means wear a laboratory coat, goggles.

3. Always use chloroform and 2-mercaptoethanol in a fume hood. When working in the fume hood, always be certain the fan is on and the sash is lowered to the correct level as indicated by the arrows on the frame and sash.

4. Whenever you have a question or concern regarding health or safety with respect to a specific procedure, consult the laboratory supervisor or the principal investigator before proceeding.

5. Extraction of good quality total RNA is vital to the production of high quality expression data. We suggest taking all the usual precautions during the preparation of the RNA to prevent degradation or contamination.

6. The Trizol or Tri-reagent method is the appropriate and eligible method used in the molecular biology laboratory for subsequent use in gene expression analysis:

    a) Homogenization. Cells collected centrifugation should be added to 1ml TRI reagent. Store homogenate for 5min at room temperature.
    b) RNA extraction. Add 0.1ml bromochloropropane or 0.2 ml of chloroform, mix vigorously. Store sample for 2-15min at room temperature. Centrifuge at 12,000g for 15min at 4oC.
    c) RNA precipitation. Transfer aqueous phase into a new tube. Add 0.5ml of isopropanol and mix, then store for 5-10min at room temperature. Centrifuge 12,000g for 8min at4-25oC.
    d) RNA wash. Mix RNA pellet with 1ml of 75% ethanol. Centrifuge at 7,50g for 5min at 4- 25oC.
    e) Solubilization. Air dry the RNA pellet for 5-10min. Dissolve by pipetting in 50-200µl of FORMAzol, 0.5%SDS, or water and incubate at 55-60oC for 10min.
    f) The absorbance of the RNA at 260nm and 280nm should be measured using quartz cuvets. The 260/280 ratio should be >1.8. An

A260 of 1.0 in a 1-cm light path is equivalent to a DNA concentration of 404g/ml. Store the RNA at -80oC.

g) An aliquot of the RNA should be analyzed by electrophoresis through a 0.3% agarose gel in denaturing condition. The prepared RNA should be treated with RNA loading buffer 1:2 at 65oC for 10min, then loaded into the wells for electrophoresis.

h) The presence of two strongly staining bands represents the 28S and 18S ribosomal RNAs, indicating intact RNA. Degradation is observed by a smear running down the length of the gel.

Change History

SOP no.	Effective Date	Significant Changes	Previous SOP no.