SOP 3.5 RNA Extraction from Blood

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Version Number 1.0

	Name	Title	Date
Author			
Authoriser			

Effective Date	
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Purpose

This SOP describes the procedure for RNA extraction from whole blood.

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 precautions

- Prior to commencing read and understand the Material Safety Data Sheets (MSDS) for all hazardous chemicals used in this procedure (chloroform, 2-mercaptoethanol, CTAB, isopropanol and isoamyl alcohol).
- Protective clothing, including laboratory coat, gloves and protective glasses, must be worn at all times when performing this procedure.
- Always use chloroform and 2-mercaptoethanol in a fume hood. When working in the fume hood, ensure the fan is on and the sash is lowered to the correct level as indicated by the arrows on the frame and sash.
- Extraction of good quality total RNA is vital to the production of high quality expression data. Follow the procedure outlined and all necessary precautions for the preparation of the RNA to prevent degradation and/or contamination.

Considerations

- RNA should be extracted as soon as is practicable after sample collection. For best results, use either fresh samples or samples that have been quickly frozen and stored at –80°C.

- 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

- Hanks balanced salt solution
- Ficoll density gradient solution
- TRI reagent
- Chloroform
- Isopropanol
- Ethanol
- RNAse free water
- A refrigerated centrifuge capable of 12,000g
- A vortex mixer
- -80^{o}C Freezer
- A spectrophotometer capable of reading 260 and 280nm/Nanodrop
- BioRad Experion/Agilent Bioanalyser 2100

Procedure: The Trizol or Tri-reagent method (Sigma Aldrich / Ambion)

1. Homogenization. Cells are isolated as follows;
   a. Dilute 10mL of blood/ cell pellet with 10mL of 1X Hanks balanced salt solution (HBSS).
   b. Layer this solution over 5mL of a ficoll density gradient centrifugation solution (for example Histopaque, Sigma Aldrich) and centrifuge in a 15mL disposable plastic centrifuge tube for 15 min at RT at 2,000g.
   c. A white band containing peripheral lymphocytes should be visible in each tube.
   d. Remove and discard the sample above this and transfer the white band to a fresh 15mL conical centrifuge tube.
   e. Wash the cells by adding 10mL of HBSS, mix thoroughly, and recover the cells by centrifugation for 10 min at RT at 2,000g.
   f. Discard the supernatant and resuspend the cell pellet in 20mL 1X TRI reagent. Store the lysate for 5min at RT (18-22°C).

2. 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 15min at 4°C.

3. 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 8min at 4-25°C.

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

5. Solubilisation: Air dry the RNA pellet for 5-10 min. Dissolve by pipetting in 50-200µl of DEPC treated RNA free water and incubate at 55-60°C for 10min.

6. 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 1-cm light path is equivalent to a RNA concentration of 40µg/mL. The RNA sample is aliquoted in RNase-free water and stored at -80°C.

7. An aliquot of the RNA from a representative sample for each batch may be also analysed by electrophoresis on a 0.3% a denaturing agarose gel. Briefly, the RNA solution should be diluted with RNA loading buffer 1:2 at 65°C for 10min and loaded into the wells and electrophoresed for 40mins at 60V. 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.

8. Total and/or mRNA quality and quantity may 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 isolation should be recorded in the study specific documentation or the data management system.

Change History

SOP Number	Effective Date	Significant Change	Previous SOP No.