Blood Derivatives: Extraction of RNA			
Category:	**Materials Handling and Documentation**		
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1.0 PURPOSE

The purpose of this SOP is to outline the standardized procedures for the automated or manual extraction of RNA from blood derivatives using Qiagen kits at Nourah's Tissue Biobank. This ensures the integrity, purity, and quality of the RNA for downstream applications in research.

2.0 SCOPE

This SOP applies to all personnel involved in the automated or manual extraction of RNA from blood samples within Nourah's Tissue Biobank, using Qiagen kits and automation systems. It covers all steps from sample preparation to the final storage of extracted RNA.

3.0 ROLES AND RESPONSIBILITIES

This SOP applies to all personnel of Nourah's Tissue Biobank members

Biobank Personnel	Responsibility
Laboratory Technicians	Responsible for performing RNA extraction using the Qiagen automated system according to this SOP, ensuring the proper handling, processing, and storage of RNA.
Biobank Manager	Responsible for overseeing RNA extraction activities, ensuring compliance with this SOP, and addressing any issues that arise.
Quality Assurance (QA) Officer	Responsible for auditing the RNA extraction process, ensuring adherence to protocols, and managing documentation.
All Personnel	Responsible for following the procedures outlined in this SOP to maintain the integrity and quality of RNA samples.

4.0 MATERIALS, EQUIPMENT, AND FORMS

Listing of the materials, equipment, and forms being used to achieve the goals of the SOP, this list will mainly contain necessary materials and, or recommendations that may be substituted by alternative or equivalent materials more suitable at the time of testing.

Material to be used	Site
Qiagen RNA extraction automation kit (compatible with the automated system)	
Qiagen automated system (e.g., QIAcube, QIAcube Connect)	
Pipettes and sterile pipette tips (for manual steps)	
RNase-free water and tubes	
Ethanol (96-100%)	
Vortex mixer	
Disposable gloves, lab coat, face shield (PPE)	
Biohazard waste containers	
Ice or cold block for RNA stabilization	
RNA storage tubes (e.g., RNase-free cryovials)	

5.0 POTENTIAL HAZARDS

In this part of the SOP, we explain the potential hazards from chemicals and methodologies used in this procedure. It will also contain information on how to handle these chemicals and the level of biosafety

Material	Safety and handling

6.0 PROCEDURES

This procedure is intended to ensure that RNA are extracted from blood samples ensuring the integrity, purity, and quality of the RNA for downstream applications in research.

6.1 PREPARATION

1. Confirm the sample's ID match the RNA extraction request, and label the new vials for the extracted RNA sample
2. If using frozen blood or blood derivatives, thaw samples on ice or in a cold block to preserve RNA integrity.
3. Mix the samples gently by inverting or vortexing to ensure homogeneity before proceeding.
4. Prepare any necessary reagents (e.g., ethanol) as indicated in the Qiagen automation protocol.
5. Wear appropriate PPE to avoid contamination of the samples.
6. Clean the workspace and automation system surfaces with an RNase decontamination solution to prevent RNA degradation Upon processing.

6.2 AUTOMATED RNA EXTRACTION

1. Power on the Qiagen automated system and perform a system check to ensure all components are functioning correctly.
2. Load the appropriate Qiagen RNA extraction kit into the automated system according to the manufacturer's instructions.
3. Transfer the appropriate volume of blood or derivative (e.g., plasma, buffy coat) into the specified sample tubes or cartridges for the automated system.
4. Ensure that samples are correctly placed in the designated positions within the automation system.
5. Select the RNA extraction protocol on the Qiagen automated system, adjusting parameters as needed for the specific sample type (e.g., whole blood, plasma).
6. If DNase treatment is required, include this step in the automated protocol to ensure removal of contaminating DNA.
7. Start the automated extraction process by initiating the program on the system's interface.
8. Monitor the system for any errors or alerts during the run, addressing issues as necessary.
9. Once the automated run is complete, carefully remove the RNA-containing tubes or cartridges from the system.
10. Inspect the eluted RNA samples for volume and quality as indicated by the system's output.
11. Aliquot the RNA into labeled RNase-free tubes and store at -80°C for long-term storage or -20°C for short-term use.
12. Log the RNA samples into the LIMS system, recording the concentration, volume, and storage location.

6.3 MANUAL RNA EXTRACTION

1. Assemble all components of the Qiagen RNA extraction kit as per the manufacturer's instructions.
2. Prepare any necessary reagents (e.g., ethanol) as indicated in the Qiagen kit protocol.
3. Transfer the appropriate volume of blood or derivative (e.g., plasma, buffy coat) into a clean, RNase-free tube.
4. Add the lysis buffer provided in the Qiagen kit to the sample, according to the protocol.
5. Vortex the sample for 30 seconds to ensure complete lysis of the cells.
6. If required, homogenize the sample using a syringe and needle or a homogenizer to ensure thorough lysis.
7. Centrifuge the lysate to remove any insoluble material and transfer the supernatant to a new tube.
8. Add the appropriate amount of ethanol to the lysate as indicated in the Qiagen protocol.
9. Mix by vortexing and transfer the mixture to the RNA binding column provided in the kit.
10. Centrifuge the column at the recommended speed to allow RNA binding to the membrane.
11. Wash the column with the buffers provided in the kit (e.g., Buffer RW1 and Buffer RPE) according to the Qiagen protocol.
12. Ensure that all traces of wash buffer are removed by centrifugation to avoid contamination.
13. If required, perform an on-column DNase treatment to remove contaminating DNA following the Qiagen DNase kit instructions.
14. This step ensures the purity of RNA for downstream applications like qPCR.
15. Elute the RNA from the column using RNase-free water or the elution buffer provided in the Qiagen kit.
16. Centrifuge the column to collect the purified RNA in a clean, RNase-free tube.
17. Aliquot the RNA into labeled RNase-free tubes and store at -80°C for long-term storage or -20°C for short-term use.
18. Log the RNA samples into the LIMS system, recording the concentration, volume, and storage location.

6.4 QUALITY CONTROL

1. Measure the concentration and purity of the RNA using nanodrop spectrophotometer
2. Assess the A260/A280 and A260/A230 ratios to ensure RNA purity (ratios between 1.8 and 2.2 are typically acceptable).
3. Check the integrity of the RNA by running an aliquot on an agarose gel

6.5 WASTE DISPOSAL

1. Dispose of any blood-contaminated materials (e.g., gloves, pipette tips) in biohazard waste containers.
2. Dispose of needles and other sharps in designated sharps containers immediately after use.
3. Clean all work surfaces and equipment with an appropriate disinfectant after processing is complete.

7.0 REFERENCES

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8.0 REVISION HISTORY

SOP No.	Date Revised	Author	Summary

9.0 APPENDICES