Blood Derivatives: Extraction of DNA

Category:	**Materials Handling and Documentation**		
SOP number:	04.004	Version	1.0
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1.0 PURPOSE

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

2.0 SCOPE

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

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 DNA extraction according to this SOP, whether manually or using the automated system, ensuring proper handling, processing, and storage of DNA.
Biobank Manager	Responsible for overseeing DNA extraction activities, ensuring compliance with this SOP, and addressing any issues that arise.
Quality Assurance (QA) Officer	Responsible for auditing the DNA 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 DNA 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 DNA extraction kit (e.g., QIAamp DNA Blood Mini Kit)	
Qiagen automated system (e.g., QIAcube, QIAcube Connect)	
Centrifuge	
RNase-free water and tubes	
Ethanol (96-100%)	
Vortex mixer	
Disposable gloves, lab coat, face shield (PPE)	
Biohazard waste containers	
DNA storage tubes (e.g., RNase-free cryovials)	
Pipettes and sterile pipette tips	

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 DNA is extracted from blood samples ensuring the integrity, purity, and quality of the DNA for downstream applications in research.

6.1 PREPARATION

1. Confirm the sample's ID match the DNA 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 DNA integrity.
3. Mix the samples gently by inverting or vortexing to ensure homogeneity before proceeding.
4. Assemble all components of the Qiagen DNA extraction kit according to the manufacturer's instructions.
5. Wear appropriate PPE to avoid contamination of the samples.
6. Clean the workspace and automation system surfaces with an RNase decontaminant solution to prevent RNA degradation Upon processing.

6.2 AUTOMATED DNA 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 DNA 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 DNA 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 DNA-containing tubes or cartridges from the system.
10. Inspect the eluted DNA samples for volume and quality as indicated by the system's output.
11. Aliquot the DNA into labeled RNase-free tubes and store at -80°C for long-term storage or -20°C for short-term use.
12. Log the DNA samples into the LIMS system, recording the concentration, volume, and storage location.

6.3 MANUAL RNA EXTRACTION

1. Assemble all components of the Qiagen DNA 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. Add the appropriate amount of ethanol to the lysate as indicated in the Qiagen protocol.
8. Mix by vertexing and transfer the mixture to the DNA binding column provided in the kit.
9. Centrifuge the column at the recommended speed to allow DNA binding to the membrane.
10. Wash the column with the buffers provided in the kit (e.g., Buffer AW1 and Buffer AW2) according to the Qiagen protocol.
11. Ensure that all traces of wash buffer are removed by centrifugation to avoid contamination.
12. Elute the DNA from the column using DNase-free water or the elution buffer provided in the Qiagen kit.
13. Centrifuge the column to collect the purified DNA in a clean, RNase-free tube.
14. Aliquot the DNA into labeled RNase-free tubes and store at -80°C for long-term storage or -20°C for short-term use.
15. Log the DNA samples into the LIMS system, recording the concentration, volume, and storage location.

6.4 QUALITY CONTROL

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

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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2. CTRnet SOPs “08.02.004 Blood Derivatives: Extraction of DNA”
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8.0 REVISION HISTORY

SOP No.	Date Revised	Author	Summary

9.0 APPENDICES