Purification of Blood Genomic DNA from RNAgard® Blood Tubes

Introduction

RNAgard® Blood Tubes (RGBT) (Cat. No. 62201-131. Biomatrica) are designed for the immediate stabilization of cellular RNA in human blood. They provide an efficient method for blood RNA collection, transport and storage at room temperature. In addition, genomic DNA in the same blood sample is preserved.

RNA purification from an RNAgard Blood Tube with the BioMaxi™ Blood RNA Purification Kit (Cat. No. 64201-601, Biomatrica) is described in the RNAgard Handbook. The following protocol can be used for purification of DNA from the same stabilized blood sample from which RNA is isolated.

Protocol: Purification of DNA from blood after precipitation of RNA

I: Precipitation of blood RNA and preparation of supernatant containing blood DNA

1. Invert the RNAgard Blood Tube 3-5 times to ensure proper mixing. Empty the contents of the tube into a clean 50 mL conical tube.
2. Pipet 3 mL of BioMaxi Precipitation Buffer (Cat. No. RP1-001-FG) into the conical tube to bring the total volume to 12 mL. Cap the tube and incubate at room temperature for 15 minutes while shaking (500-750 rpm).
3. Vortex the conical tube at maximum speed for at least 30 seconds, ensuring that the solution travels to the top of the tube to achieve proper mixing of the contents.
4. Centrifuge the tube at room temperature for 10 minutes at 9,000 x g in a fixed rotor, or 30 minutes at 4,500 x g in a swinging bucket rotor.
5. Carefully decant the ~12 mL of supernatant containing DNA into a labeled tube. Use the pellet containing the RNA for purification as described in the RNAgard Blood Handbook. The DNA-containing supernatant can be stored at 4°C for at least one month, or immediately processed for DNA purification following any of the protocols described below.

II: Blood DNA purification

1. Aliquot the desired volume of the DNA-containing supernatant from the above precipitation protocol (Section I, Step 5).
2. Perform DNA purification using one of the following 5 methods.

A. QIAamp® DNA Blood Mini Kit (Cat. No. 51104, QIAGEN)

1. Pipet 20 µL QIAGEN Protease into the bottom of a 1.5 mL microcentrifuge tube.
2. Transfer 350 µL of DNA-containing supernatant from the above precipitation protocol (Section I, Step 5) to the microcentrifuge tube (= 80 - 85 µL whole blood) and pulse-vortex to mix sample.
3. Process DNA purification starting from Step #4 as described in the “Protocol: DNA Purification from Blood or Body Fluids (Spin Protocol)” using the QIAamp DNA Blood Mini Kit.

Note: Larger sample volumes can be processed by increasing accordingly the volumes of QIAGEN Protease, AL Buffer and EtOH to be added to the sample before processing through the silica column.

B. QIAamp® DNA Blood Midi Kit (Cat. No. 51183, QIAGEN)

1. Pipet 100 µL QIAGEN Protease into the bottom of a 15 mL centrifuge tube.

2. Transfer 1.1 mL of DNA-containing supernatant from the above precipitation protocol (Section I, Step 5) to the centrifuge tube (~240 µL of whole blood) and pulse-vortex to mix sample.

3. Add 1.1 mL of Buffer AL and continue with step # 3 as described in the "Protocol: Purification of DNA from Whole Blood Using the QIAamp Blood Midi Kit (Spin Protocol)".

Note: Larger sample volumes can be processed by increasing accordingly the volumes of QIAGEN Protease, AL Buffer and EtOH to be added to the sample before processing through the silica column.

C. QIAamp® DNA Blood Maxi Kit (Cat. No. 51192, QIAGEN)

1. Pipet 500 µL QIAGEN Protease into the bottom of a 50 mL centrifuge tube.
2. Transfer 5.5 mL of DNA-containing supernatant from the above precipitation protocol (Section I, Step 5) to the centrifuge tube (~1.2 mL whole blood and pulse-vortex to mix sample).
3. Add 5.5 mL of Buffer AL and continue with Step # 3 as described in the "Protocol: Purification of DNA from Whole Blood Using the QIAamp Blood Maxi Kit (Spin Protocol)".

Note: Larger sample volumes can be processed by increasing accordingly the volumes of QIAGEN Protease, AL Buffer and EtOH to be added to the sample before processing through the silica column.

D. MagNa Pure Compact System (Roche Applied Science)

1. Transfer 1 mL DNA-containing supernatant from the above precipitation protocol (Section I, Step 5) to a sample tube provided in the MagNA Pure Compact RNA Isolation Kit I-Large Volume (Cat. No. 04802993001, Roche Applied Science).
2. Place the sample tube in the MagNA Pure Compact tube rack and start the "DNA_Blood_1000" protocol as described in the MagNA Pure Compact Nucleic Acid Isolation Kit I-Large Volume manual.

E. Conventional Phenol/Chloroform DNA Extraction

Perform conventional phenol/chloroform DNA extraction with the desired volume of DNA-containing supernatant from Section I, Step 5.