

## **RNA Extraction**

### **I) Purpose**

This protocol describes the extraction, purification, and assay of total RNA from human peripheral blood collected in the QIAGEN Paxgene tube.

### **II) Principle**

The PAXgene Blood RNA Kit is for the purification of total RNA from human whole blood collected in a PAXgene Blood RNA Tube. Purification begins with a centrifugation step to pellet nucleic acids in the PAXgene Blood RNA Tube. The pellet is washed and resuspended, and incubated in optimized buffers together with proteinase K to bring about protein digestion. An additional centrifugation through the PAXgene Shredder spin column is carried out to homogenize the cell lysate and remove residual cell debris, and the supernatant of the flow-through fraction is transferred to a fresh microcentrifuge tube. Ethanol is added to adjust binding conditions, and the lysate is applied to a PAXgene RNA spin column. During a brief centrifugation, RNA is selectively bound to the PAXgene silica membrane as contaminants pass through. Remaining contaminants are removed in several efficient wash steps. Between the first and second wash steps, the membrane is treated with DNase I to remove trace amounts of bound DNA. After the wash steps, RNA is eluted in elution buffer and heat-denatured.

### **III) Procedure**

1. Centrifuge the PAXgene Blood RNA Tube for 10 minutes at 4000 x g using a swing-out rotor.
2. Remove the supernatant by decanting or pipetting. Add 4 ml RNase-free water to the pellet, and close the tube using a fresh secondary BD Hemogard closure.
3. Vortex until the pellet is visibly dissolved, and centrifuge for 10 minutes at 4000 x g using a swing-out rotor. Remove and discard the entire supernatant.
4. Add 350 µl resuspension buffer (Buffer BR1), and vortex until the pellet is visibly dissolved.
5. Pipet the sample into a 2 ml processing tube.
6. Load the open 2 ml processing tubes containing sample into the QIAcube shaker.
7. Select the "PAXgene Blood RNA Part A" protocol, and start the protocol.
8. After the "PAXgene Blood RNA Part A" protocol is finished, Close the lids of all 1.5 ml tubes containing the purified RNA. Transfer the 1.5 ml tubes into the corresponding positions in the QIAcube shaker adapter.
9. Select the "PAXgene Blood RNA Part B" protocol, and start the protocol.
10. After the "PAXgene Blood RNA Part B" program is finished, immediately place the microcentrifuge tubes containing the purified RNA on ice.

If the RNA samples will not be used immediately, store at  $-20^{\circ}\text{C}$  or  $-70^{\circ}\text{C}$ .

#### **IV) Biohazard Considerations**

This protocol describes the extraction of RNA from human blood. It is important to consider the potential for infection or disease transmission by materials used in this procedure that contact the sample and/or the homogenate. If using potentially infectious materials, conduct this work in an appropriate biosafety lab following OSHA and institutional protocols for the handling of biohazardous materials. After working with biohazardous materials, thoroughly decontaminate the workspace and instruments according to institutional protocol. Consider wiping surfaces with undiluted bleach and/or ethanol after the protocol is complete. Dispose of all biohazardous or potentially infectious waste according to institutional protocol. RNase contamination should be avoided.

#### **V) Analyze Data**

Analyze data using Agilent's Bioanalyzer 2100 NANO and Small RNA chips for total RNA and miRNA qualification and the NANO DROP for quantification.

#### **VI) Reference**

See more details in the related websites of the QIAGEN and Agilent's Technologies.