Promega

Product Application

Total RNA Purification from Blood with the Maxwell® RSC miRNA Tissue Kit

Automated purification of total RNA, including miRNA, from blood samples.

Kit: Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)

Analyses: UV Absorbance, QuantiFluor® RNA Dye, and RT-qPCR with TaqMan® miRNA Assay

Sample Type(s): Blood

Input: Up to 2.5ml blood collected in K2 EDTA tube

Materials Required:

- Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Cell Lysis Buffer (Cat.# A7933)
- Vortex Mixer
- Centrifuge

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM441, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Protocol:

1. Combine one volume of fresh blood with 3 volumes of Cell Lysis Buffer (e.g., 2.5ml blood + 7.5ml Cell Lysis Buffer) in an appropriately sized tube. Invert 5-6 times to mix.
2. Incubate for 10 minutes at room temperature, inverting 2 times to mix during the incubation.
3. Centrifuge at 3,000 *x g* for 10 minutes to pellet white blood cells (WBC).
4. Remove and discard as much of the supernatant as possible without disturbing the visible pellet.
5. Briefly centrifuge to collect residual liquid at the bottom of the tube. Remove and discard the supernatant.
6. Add 200µl of chilled Homogenization Solution with 2% 1-Thioglycerol to the WBC pellet and vortex to resuspend.
7. Continue with step 2 on page 5 of the Maxwell® RSC miRNA Tissue Kit Technical Manual (TM441) for manual sample pre-processing.
8. Process the samples on the Maxwell® RSC Instrument using the miRNA Tissue Kit method.

Results:

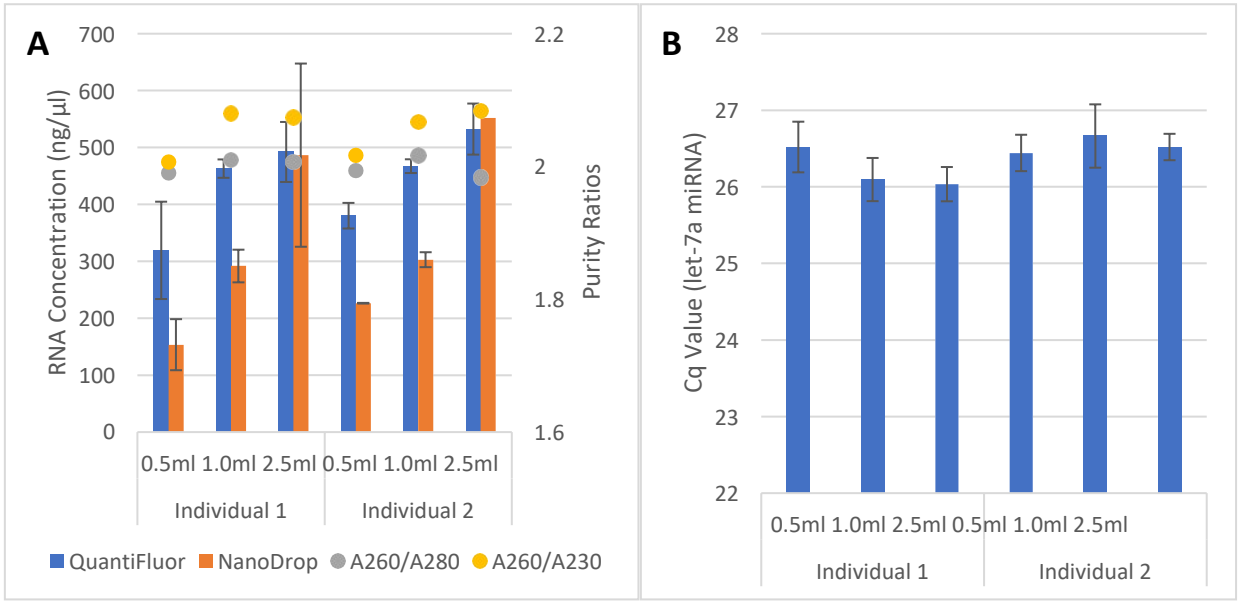

Figure 1. Differential lysis followed by centrifugation was used to generate a white blood cell (WBC) pellet from 0.5, 1.0, and 2.5ml blood from two individuals according to the protocol above. Total RNA was extracted from the WBC pellet following the Maxwell® RSC miRNA Tissue protocol. Panel A shows RNA concentration that was measured via fluorescence with the QuantiFluor® RNA System (Cat.# E3310), and by UV absorbance with the NanoDrop® 8000 Spectrophotometer. A260/A280 and A260/A230 purity ratios were also measured by NanoDrop® absorbance and are shown with gray and yellow circles, respectively. For RNA concentration and purity ratios, mean ± SD of n=3 is shown. Panel B shows Cq values for amplification of the let-7a miRNA in 2-step RT-qPCR. cDNA was generated on the GeneAmp 9700 PCR System using the TaqMan® miRNA Reverse Transcription Kit under the following thermal conditions: 16°C for 30 minutes; 42°C for 20 minutes; 85°C for 5 minutes; 4°C hold. qPCR was then completed using the GoTaq® 2-Step RT-qPCR System (Cat.# A6120) on the Bio-Rad CFX™ 96 Real-Time PCR Detection System under the following thermal conditions: 95°C for 2 minutes; 40 cycles of [95°C for 10 seconds; 60°C for 60 seconds]. Mean ± SD of n=3 is shown.