Promega

Product Application

Automated Total RNA Purification from Blood on a Mitra® Microsampler

Purify Total RNA, including miRNA, from whole blood collected on a Mitra® microsampler using the Maxwell® RSC miRNA Plasma and Serum Kit and the Maxwell® RSC Instrument.

Kit: Maxwell® RSC miRNA Plasma and Serum Kit (Cat.# AS1680)

Analyses:

- Dye-based quantitation
- RT-qPCR
- TapeStation

Sample Type(s): Blood samples collected using Neoteryx® Mitra® microsampler device

Input: One Mitra® microsampler tip

Materials Required:

- Maxwell® RSC miRNA Plasma and Serum Kit (Cat.# AS1680)
- Maxwell® RSC Instrument (Cat.# AS4500)
- CW Spin Baskets (Cat.# AS8101)
- CW Microfuge Tubes (Cat.# AS8201)
- Heat block

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 TM546, available at: **www.promega.com/protocols**

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

Protocol:

1. Place one Mitra® microsampler tip into a CW Spin Basket inside a CW Microfuge Tube.
2. Add 100µl of Nuclease-Free Water, 230µl of Lysis Buffer C and 80µl of Proteinase K to the CW Spin Basket. Vortex for 5-10 seconds.
3. Ensure Mitra® microsampler tip is fully submerged, then incubate at 37°C for 15 minutes.
4. Centrifuge at maximum speed for 5 minutes to collect lysate at the bottom of the tube.
5. Transfer the full lysate to well #1 of the Maxwell® RSC miRNA Plasma and Serum Kit cartridge.
6. Prepare Maxwell® cartridge.
    a. Place an elution tube with 50µl of Nuclease-Free Water onto the sample rack.
    b. Place a plunger in well #8 of the cartridge.
    c. Add 10µl of blue DNase I Solution (prepared as indicated in TM546) to well #4 of the Maxwell® RSC cartridge (well #4 contains yellow reagent). After the blue DNase I Solution is added, the reagent in well #4 will be green.
    d. Select the Maxwell® RSC miRNA Plasma and Serum method and run.

Results:

Total RNA, including miRNA, was successfully purified from a 30µl Mitra® microsampler tip using the method described above.

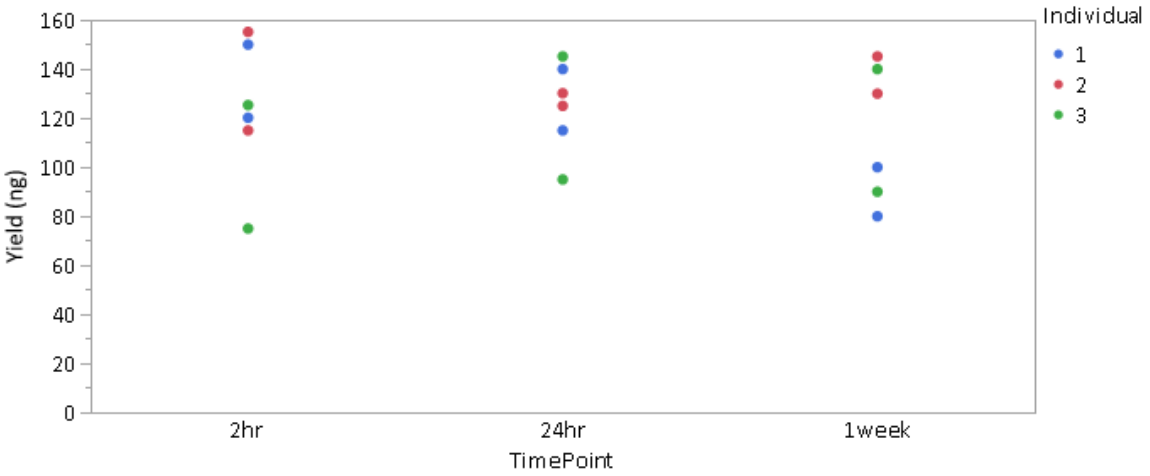

Figure 1. RNA yield of purified RNA eluates. RNA was purified from a 30µl Mitra® microsampler tip in duplicate from three individuals using the method described above. After sample collection, the Mitra® microsampler was allowed to dry 2 hours, 24 hours or 1 week before RNA purification. RNA was quantified using an RNA-binding fluorescent dye (QuantiFluor® RNA System, Cat.# E3310).

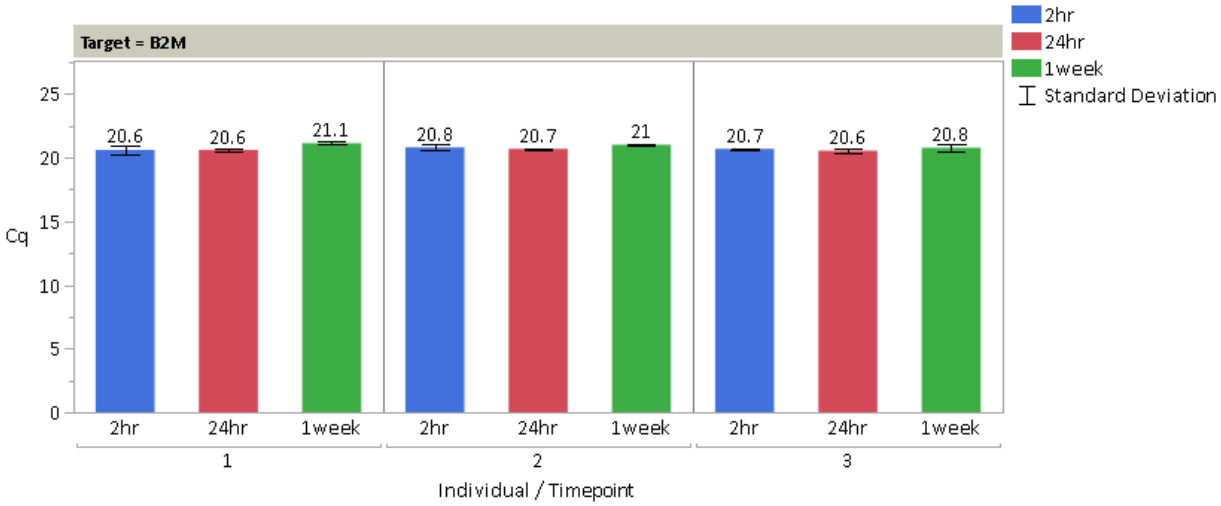

Figure 2. RNA amplifiability of purified RNA eluates. Total RNA was purified from a 30µl Mitra® microsampler tip in duplicate from three individuals using the method described above. After sample collection, the Mitra® microsampler was allowed to dry 2 hours, 24 hours or 1 week before RNA purification. Eluates were amplified with the GoTaq® 1-Step RT-qPCR System (Cat.# A6020) and RNA-specific primers targeting β2M mRNA. Data represents the average Cq value and standard deviation for duplicate purifications amplified in duplicate.

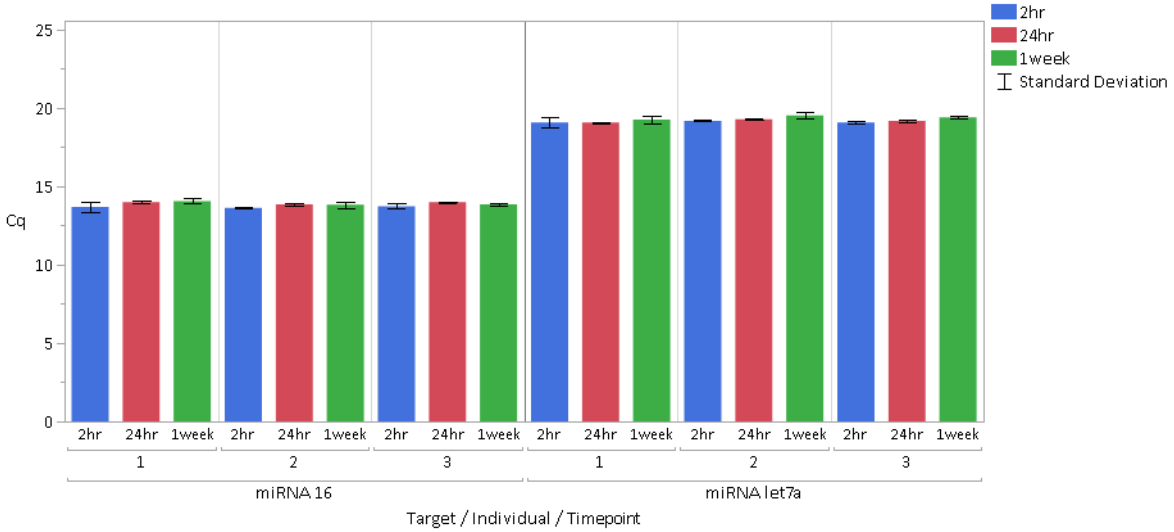

Figure 3. miRNA detection of purified RNA eluates. Total RNA was purified from a 30µl Mitra® microsampler tip in duplicate from three individuals using the method described above. After sample collection, the Mitra® microsampler was allowed to dry 2 hours, 24 hours or 1 week before RNA purification. Eluates were reverse transcribed with TaqMan® miRNA 2-step RT-qPCR Assays for miRNAs let-7a (Assay ID 000377) and mi16 (Assay ID 000391) and amplified using GoTaq® Probe qPCR Master Mix (A6101) with TaqMan® miRNA probe and primer sets. Data represents the average Cq value and standard deviation for duplicate purifications with a single reverse transcriptase reaction amplified in duplicate.

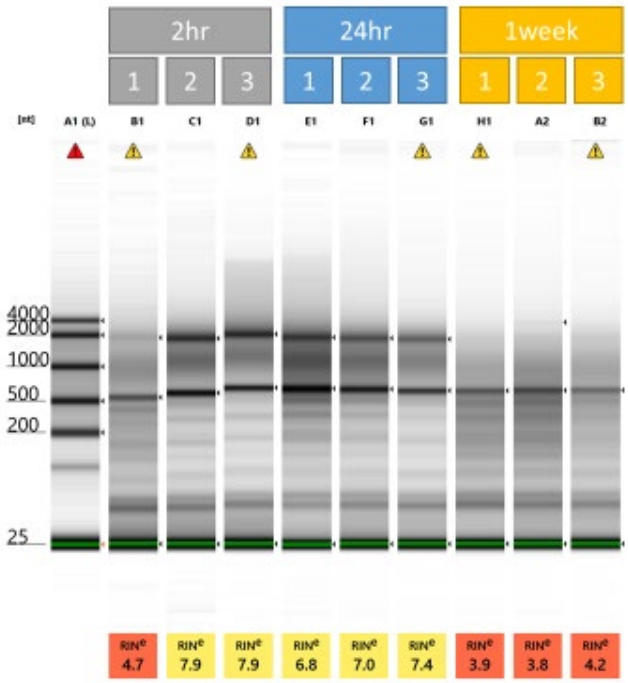

Figure 4. TapeStation electrophoresis of purified RNA eluates. Total RNA was purified from a 30µl Mitra® microsampler tip in duplicate from three individuals using the method described above. After sample collection, the Mitra® microsampler was allowed to dry 2 hours, 24 hours or 1 week before RNA purification. One purification replicate from each individual and timepoint was analzyed using the High Sensitivity RNA ScreenTape Assay on the 4200 TapeStation System.