Promega

Product Application

Automated RNA Purification from Blood on a Mitra® Microsampler Treated with RNA Stabilizer

Purify Total RNA, including miRNA, from whole blood collected on a Mitra® microsampler treated with RNA stabilizer using the Maxwell® RSC simplyRNA Blood Kit and the Maxwell® RSC Instrument.

Kit: Maxwell® RSC simplyRNA Blood Kit (Cat.# AS1380)

Analyses:

- Dye-based quantitation
- TapeStation™
- RT-qPCR

Sample Type(s): Blood samples collected using Neoteryx® Mitra® microsampler devices treated with RNA stabilizer

Input: One Mitra® microsampler tip

Materials Required:

- Maxwell® RSC simplyRNA Blood Kit (Cat.# AS1380)
- Maxwell® RSC Instrument (Cat.# AS4500)
- CW Spin Baskets (Cat.# AS8101)
- CW Microfuge Tubes (Cat.# AS8201)

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 TM417, 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. Prepare Homogenization Solution by adding 20µl of 1-Thioglycerol per 1ml of Homogenization Solution.
3. Add 200µl of Homogenization Solution with 1-Thioglycerol, 200µl of Lysis Buffer, and 25µl of Proteinase K to the CW Spin Basket. Vortex 5-10 seconds.
4. Ensure Mitra® microsampler is fully submerged, then incubate at room temperature for 10 minutes.
5. Centrifuge at maximum speed for 5 minutes to collect lysate at the bottom of the tube.
6. Transfer the full lysate to well #1 of the Maxwell® RSC simplyRNA Blood Kit cartridge.
7. Add 10µl of blue DNase I solution (prepared as described in Technical Manual TM417 Section 3.A.) to well #4 of the Maxwell® RSC cartridge.
8. Prepare Maxwell® cartridge according to the Technical Manual (TM417, Section 4.B.).
9. Select the Maxwell® RSC simplyRNA Blood method and run.

Results:

Total RNA, including miRNA, was successfully purified from 30µl Mitra® microsampler tips with RNA stabilizer as described above.

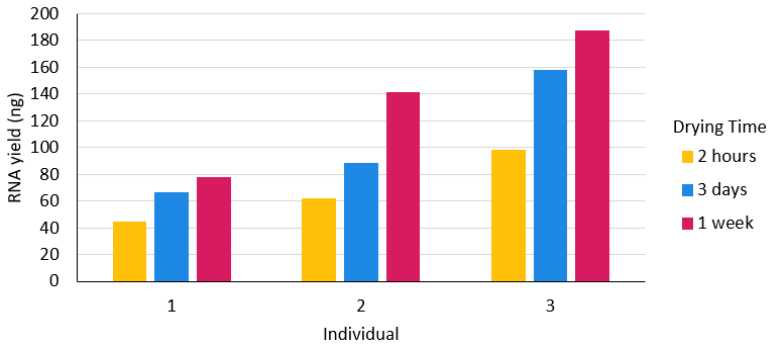

Figure 1. RNA yield of purified RNA eluates. RNA was purified from 30µl Mitra® microsampler tips in duplicate from three individuals using the method described above. After sample collection, the Mitra® microsampler was dried for 2 hours, 3 days, or 1 week before RNA purification. RNA was quantified using an RNA-binding fluorescent dye (QuantiFluor® RNA System, Cat.# E3310). Average of n=2 shown.

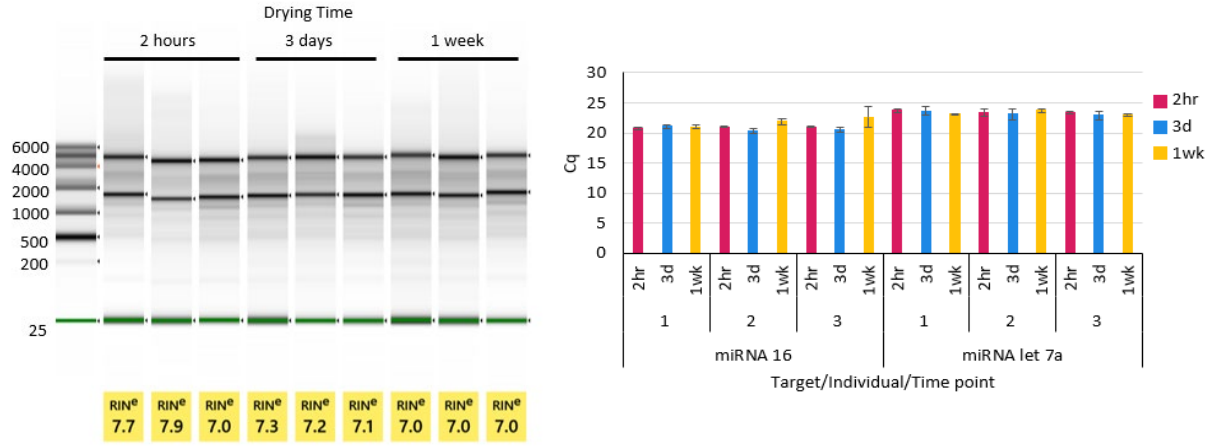

Figure 2. RNA and miRNA detection by TapeStation Electrophoresis and RT-qPCR. Total RNA was purified from 30µl Mitra® microsampler tips in duplicate from three individuals using the method described above. After sample collection, the Mitra® microsampler was allowed to dry 2 hours (2hr), 3 days (3d) or 1 week (1wk) before RNA purification. **(Left)** One purification replicate from each individual and timepoint was analzyed using the High Sensitivity RNA ScreenTape Assay on the 4200 TapeStation™ System (Agilent). **(Right)** RNA eluates from each individual were reverse transcribed with TaqMan® miRNA 2-step RT-qPCR Assays for miR-16 (Assay ID 000391) and let-7a (Assay ID 000377) and amplified using GoTaq® Probe qPCR Master Mix (A6101) with TaqMan® miRNA probe and primer sets. Single reverse transcriptase reactions were prepared with each purification duplicate, and each resulting cDNA was amplified in duplicate. Data represents the average Cq value and standard deviation of the mean of the two sets of duplicate qPCR reactions derived from two cDNA replicates per individual.