Promega

Product Application

Automated Total RNA Purification from Stabilized Saliva

Purify total RNA, including miRNAs, from saliva collected in OMNIgene•ORAL collection devices with the Maxwell® RSC miRNA Tissue Kit on Maxwell® RSC Instruments.

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

Analyses: Absorbance, dye-based quantitation, RT-qPCR, TapeStation ScreenTape Analysis

Sample Type: Human saliva collected in OMNIgene•ORAL collection devices

Input: 400µl

Materials Required:

- Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)
- OMNIgene•ORAL collection devices (DNA Genotek, OME-505)
- Maxwell® RSC Instrument (Cat.# AS4500) or Maxwell® RSC 48 Instrument (Cat.# AS8500)

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:

Note: The protocol does not utilize Homogenization Solution or Lysis Buffer included with the kit.

1. Add 400µl of saliva collected in OMNIgene•ORAL collection devices (stabilized saliva) to a 1.5ml tube.
2. Add 200µl of Lytic Enhancer and 30µl of Proteinase K (PK) Solution.
3. Mix by vortexing for 20 seconds.
4. Incubate for 10 minutes at room temperature.
5. During the incubation, prepare Maxwell® RSC Cartridges.
    a. Add 10µl of DNase I Solution (prepared as described in Technical Manual TM441) to well #4.
    b. Add 60µl of Nuclease-Free Water to each Elution Tube and place in the deck tray with lid open.
    c. Add a plunger to well #8.
6. Following incubation, transfer the entire lysate volume to well #1 of the Maxwell® RSC cartridge.
7. Purify total RNA using the miRNA Tissue protocol on the Maxwell® RSC Instrument or Maxwell® RSC 48 Instrument.

Results:

Total RNA including miRNAs was successfully purified from stabilized saliva in OMNIgene•ORAL collection devices. The purified RNA was of good quality, with sufficient yield and detection of mRNA and miRNA targets by RT-qPCR.

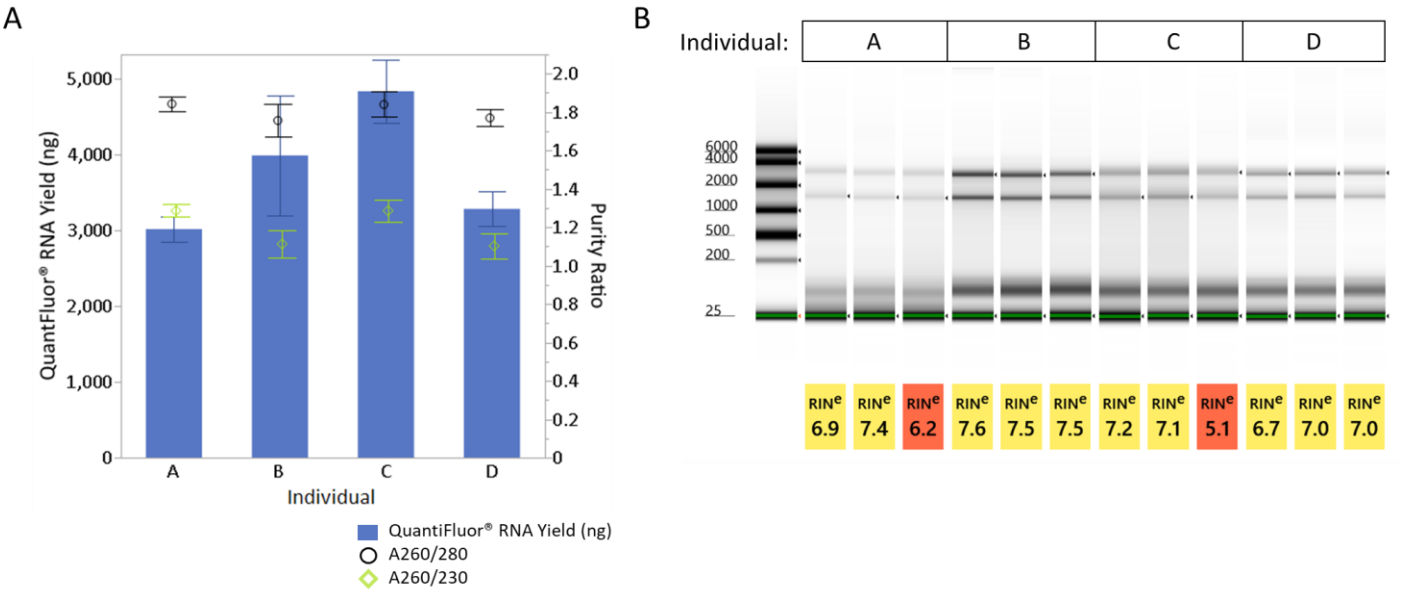

Figure 1. Yield and quality of RNA purified from stabilized saliva. Saliva samples were collected from four individuals in OMNIgene•ORAL collection devices, according to manufacturer's instructions, and incubated overnight at room temperature. RNA was purified in triplicate from each sample according to the protocol above. **A.** RNA yield and purity. RNA concentration was measured with the QuantiFluor® RNA System (Cat.# E3310) using the high standard protocol and read on a Quantus™ Fluorometer (Cat.# E6150). Yield was calculated by multiplying concentration by eluate volume. Purity ratios were measured by absorbance using a NanoDrop™ ND8000 spectrophotometer. Yield, blue bars; A260/280, black circles; A260/230, green diamonds. Mean ± standard deviation is shown for each measurement (n=3). **B.** RNA integrity. Undiluted RNA samples were analyzed by electrophoresis on RNA ScreenTapes with an Agilent TapeStation 4200, according to manufacturer's instructions. Gel image is shown. Ladder and associated sizing is shown at the left of the image. RIN^e values are likely negatively impacted by copurification of bacterial RNA.

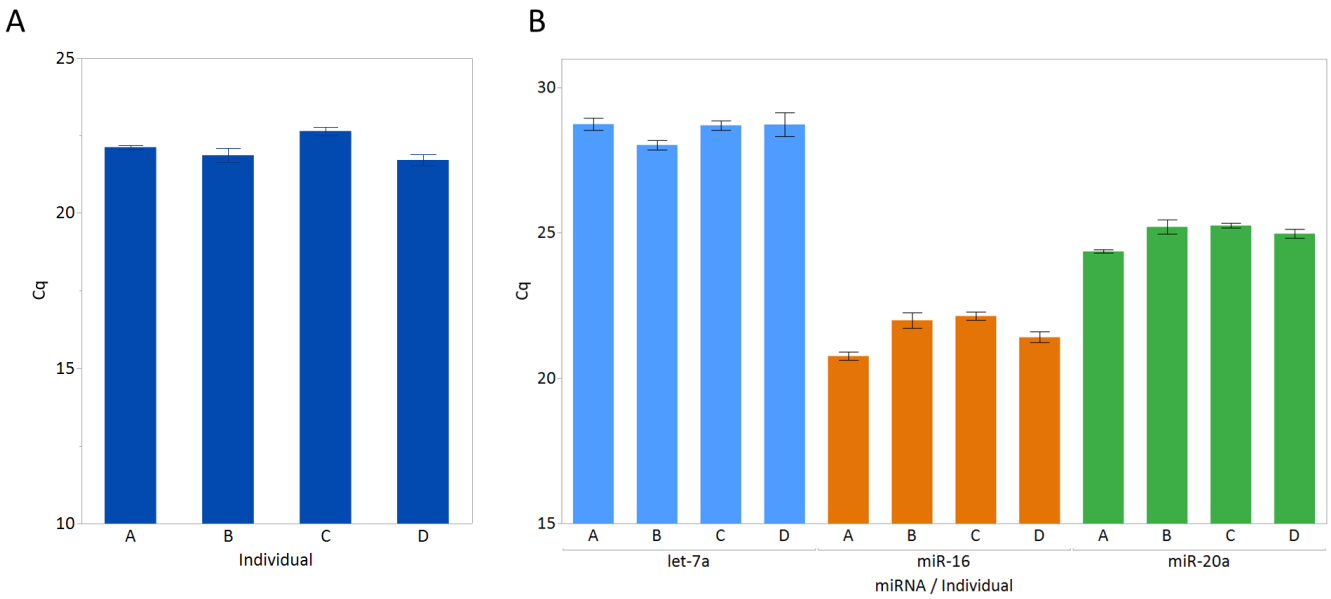

Figure 2. Amplification of mRNA and miRNA targets from RNA purified from stabilized saliva. A. Amplification of human β2 microglobulin (β2M) mRNA. RNA was diluted to 25ng/µl and 2µl was used for 1-step-RT-qPCR with β2M-specific primers using GoTaq® 1-Step RT-qPCR System (Cat.# A6020). Mean Cq ± standard deviation is shown (n=6, three purification replicates amplified in duplicate). **B.** Amplification of miRNA targets. cDNA was synthesized with TaqMan microRNA Reverse Transcription Kit (ThermoFisher Scientific) and RT primers from the TaqMan microRNA assays (ThermoFisher Scientific) corresponding to each miRNA: Human let-7a, miR-16 and miR-20a. miRNA targets were amplified using GoTaq® Probe 1-Step RT-qPCR Master Mix (Cat.# A6120) and TaqMan microRNA Assay primer/probe mix specific for each miRNA. Mean Cq ± standard deviation is shown for each miRNA indicated (n=6, three purification replicates amplified in duplicate).