Promega

Product Application

Automated purification of RNA from Human Saliva Collected in Oragene® Collection Kits

Purify RNA from human saliva collected in Oragene® RE-100 devices using the Maxwell® RSC Instrument and Maxwell® RSC miRNA Tissue Kit.

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

Analyses: UV absorbance, dye-based quantitation

Sample Type(s): Human saliva in Oragene®●RNA collection kit

Input: 400µl of total sample (200µl saliva)

Materials Required:

- Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Oragene®●RNA collection kit (DNA Genotek RE- 100)
- Heat Block capable of 90°C
- Vortex mixer

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:

2ml of saliva was collected with the Oragene®●RNA collection kit from six individuals. Samples were allowed to sit for 5 days at room temperature. Samples were incubated at 50°C for 1hr. Samples were then processed as follows:

1. Transfer a 400µl aliquot of sample to a 1.5 ml microtube.
2. Incubate the aliquot at 90°C for 15 minutes, then cool to room temperature.
3. Add 1/25th volume (16µl) of neutralizer solution. Vortex to mix thoroughly.
4. Incubate on ice for 10 minutes.
5. Centrifuge at 13,000 × *g* for 3 minutes.
6. Remove the supernatant being careful not to disrupt the pellet and transfer to well #1 of the Maxwell® RSC cartridge placed into the sample rack.
7. Place one of the supplied elution tubes into the sample rack and add 50µl of Nuclease-Free Water for each sample.
8. Place the plunger in the indicated position of each cartridge.
9. Add 10µl of DNase I to well #4 of the Maxwell® RSC cartridge.
10. Select the miRNA Tissue method on the Maxwell® RSC instrument.

Results:

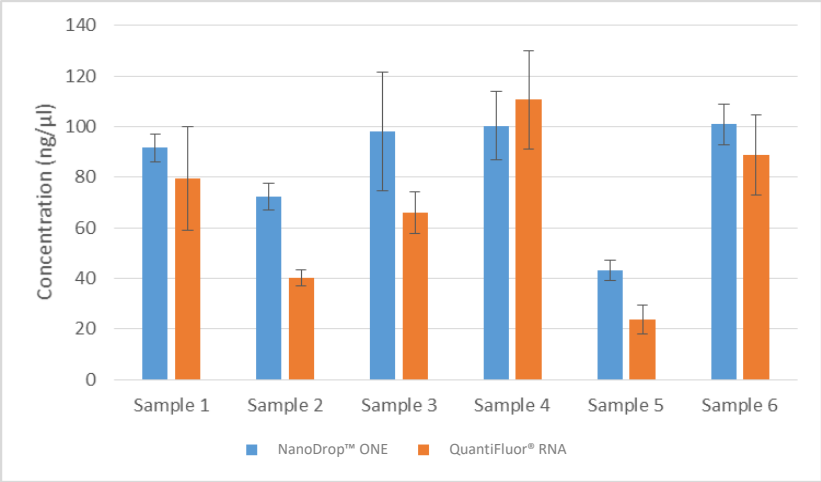

Figure 1. Analysis of RNA from saliva samples. Purified DNA was analyzed using the NanoDrop ONE instrument (Thermo Fisher Scientific) and the QuantiFluor® RNA System (Cat.# E3310) on the Quantus™ Fluorometer (Cat.# E6150). ~32µl of eluate was recovered.RNA concentrations ranged from 43.2 – 100.9 ng/µl based on NanoDrop™ and 23.7 – 110.7 ng/µl based on QuantiFluor®. Results are an average of triplicate purifications of each sample. Standard deviations are shown.

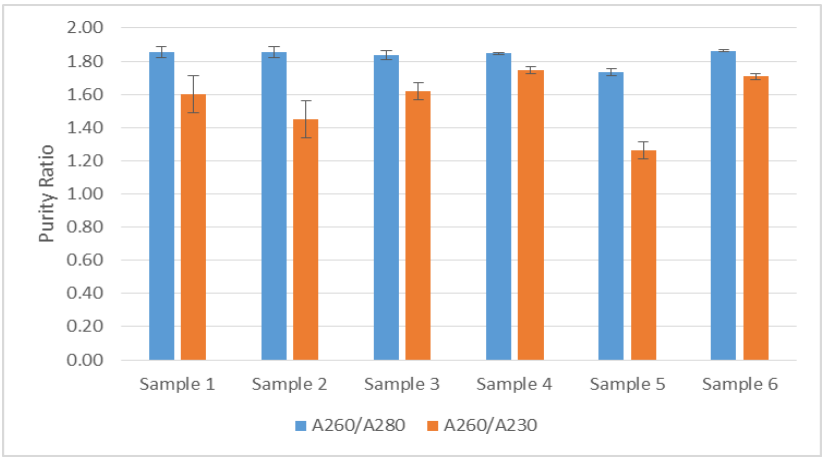

Figure 2. Purity of RNA from saliva samples. A260/A280 ratios averaged 1.83 with a range from 1.73 – 1.86. A260/A230 ratios averaged 1.56 with a range from 1.26 – 1.71.

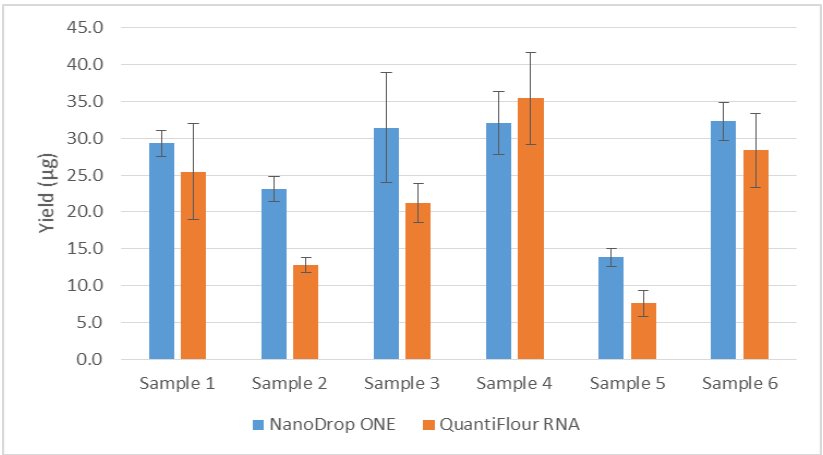

Figure 3. Total yield of RNA per 2ml of saliva collected. Total RNA yield based on NanoDrop averaged 27.0 µg and ranged from 13.8 – 32.3 µg. Total RNA yield based on QuantiFluor® averaged 21.8 µg and ranged from 7.6 – 35.4 µg.