Promega

Product Application

RNA Purification from Mint Leaves and Roots using the Maxwell® RSC System

RNA purification from mint leaves and roots using the Maxwell® RSC Plant RNA Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC Plant RNA Kit (Cat.# AS1500)

Analyses: NanoDrop, QuantiFluor® RNA System (Cat.# E3310)

Sample Type(s): Mint leaves and roots

Input: 100mg and 50mg

Materials Required:

- mint plant
- liquid nitrogen
- mortar and pestle
- Biospec Tissue-Tearor

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 TM459, available at: www.promega.com/protocols

or contact Technical Services at: techserv@promega.com

Protocol:

1. Grind mint leaves and roots to a fine powder in liquid nitrogen with a mortar and pestle in bulk and freeze at –80°C.
2. Weigh and pool samples (N=4). Transfer 200mg or 400mg of each respective sample type to a 15ml conical tube.
3. Add 2400µl of chilled 1-Thioglycerol/Homogenization solution to each tube.
4. Homogenize with a small tissue homogenizer for 30–60 seconds, and then place on ice. If foaming occurs, let the sample settle on ice and homogenize in 15- to 30-second increments. Place the sample on ice.
5. With a wide-bore pipette, transfer 400µl of homogenate to a 1.5ml microcentrifuge tube. Prepare enough tubes for N=3. Discard the remainder of the homogenate.
6. Add 200µl of Lysis Buffer to 400µl of homogenate and vortex vigorously for 15 seconds to mix.
7. Incubate at room temperature for 10 minutes.
8. Centrifuge at maximum speed for 2 minutes.
9. Transfer as much of the supernatant lysate as possible to well #1 of the Maxwell® cartridge (corresponding to 50mg or 100mg starting input per cartridge).
10. Add 5µl of DNase I solution to well #4 of the Maxwell® cartridge and 50µl of Nuclease-Free Water to elution tubes.

Results:

RNA was successfully isolated from 50mg and 100mg of mint leaves and roots using the Maxwell® RSC Plant RNA Kit. Following RNA purification, concentration was measured by absorbance (NanoDrop) and fluorescent dye-based (QuantiFluor® RNA System) quantitation methods (Figure 1). Purity was also determined (Figure 2). RNA was run on a standard 1% agarose gel in TE Buffer to visualize RNA integrity (Figure 3).

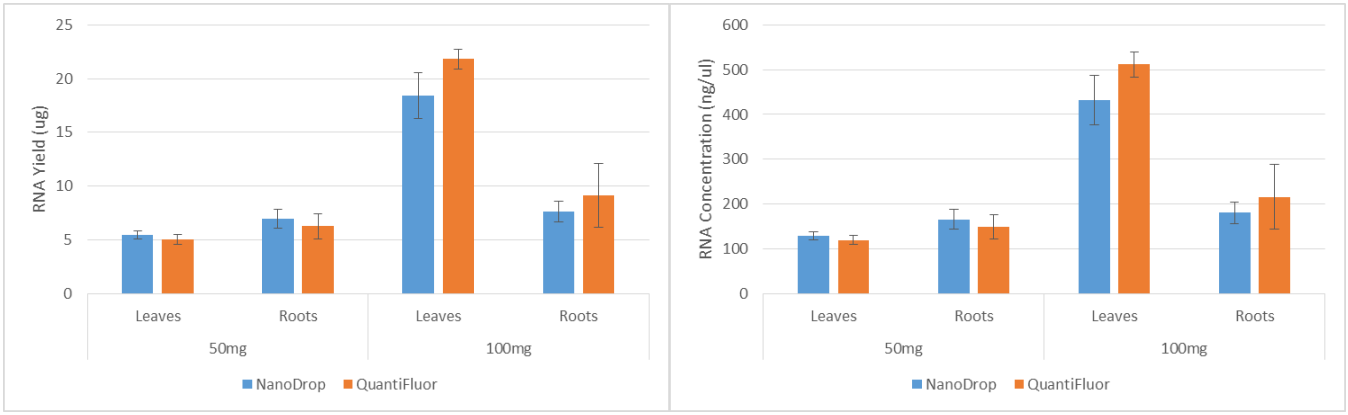

Figure 1. Yield and concentration of RNA purified from mint leaves and roots. Data is based on absorbance (NanoDrop) and fluorescent dye-based (QuantiFluor® RNA System) quantitation methods. N=3. Graphs show data from 50mg and 100mg inputs. **Left panel,** yield. **Right panel,** concentration.

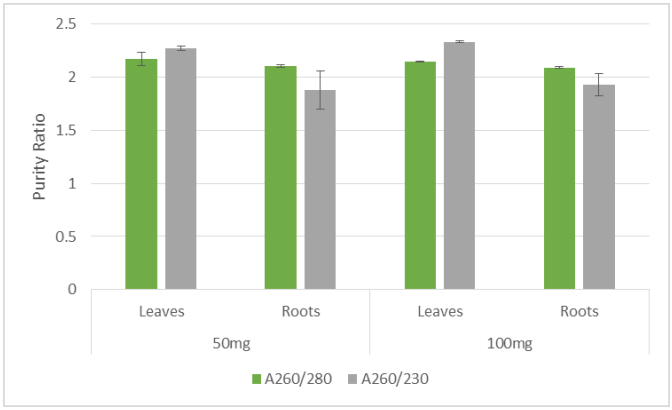

Figure 2. **Purity of RNA extracted from mint leaves and roots based on NanoDrop.** N=3. Graphs show data from 50mg and 100mg inputs.

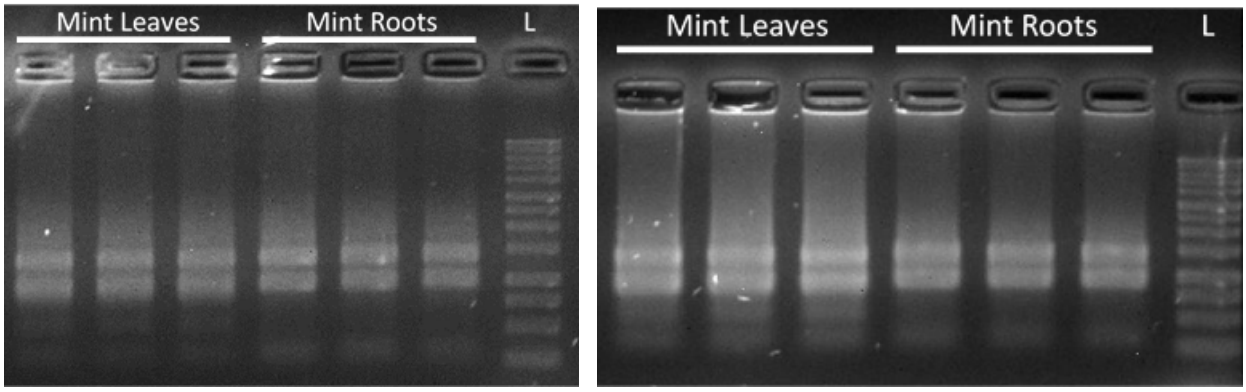

Figure 3. 1% agarose gels run in 1X TBE Buffer at 70V for 35 minutes. Two distinct bands show the 16S and 23S ribosomal RNA of every sample. **Left panel,** 50mg input. **Right panel,** 100mg input.