Promega

Product Application

RNA Purification from Plant Leaf and Seed using the Maxwell® HT simplyRNA Kit

Automated purification of RNA from plant leaf and seed using the custom Maxwell® HT simplyRNA Kit on the KingFisher™ Flex™ Automation Platform in 1 hour 30 minutes for 96 samples.

Kit: Maxwell® HT simplyRNA Kit, Custom (Cat.# AX7890)

Analyses: UV absorbance and QuantiFluor® quantitation, qPCR amplification, gel electrophoresis

Sample Type(s): corn, soybean and *Arabidopsis* leaves, corn seed

Input : 4mm leaf punch, 20–50mg plant leaf, or a single seed

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, contact Technical Services at: techserv@promega.com

Materials Required:

- Maxwell® HT simplyRNA, Custom (Cat.# AX7890)
- KingFisher Flex, with 96 Deep-well Head
- deep well 96 plates (Thermo Scientific Cat.# 95040460)
- KingFisher 96 Tip Comb for DW Magnets (Thermo Scientific Cat.# 97002534)
- 80% ethanol
- Bead-beating instrument and consumables (e.g., Fastprep®-24, MP Biomedicals)

Solution Preparation:

Homogenization Solution: To prepare a working solution, add 20µl of 1-Thioglycerol per milliliter of Homogenization Solution. 1-Thioglycerol is viscous, so careful pipetting is required for accurate measurement. A volume of 200µl of 1-Thioglycerol/Homogenization Solution is needed for each sample. Before use, chill the 1-Thioglycerol/Homogenization Solution on ice or at 2–10°C.

DNase I: Add 275µl of Nuclease-Free Water to the vial of lyophilized DNase I. Invert to rinse DNase off the underside of the cap and swirl gently to mix; do not vortex. Dispense the DNase I solution into single-use aliquots in nuclease-free tubes. Store reconstituted DNase I at –20°C. Do not freeze-thaw reconstituted DNase I more than three times.

Plate Preparation:

- Plate 1 = Tip Plate
  - Add KingFisher™ 96 Tip Comb for DW Magnets.
- Plate 2 = Elution Plate
  - Add 110µl of Nuclease Free Water per well.
- Plate 3 = 80% EtOH
  - Add 200µl 80% EtOH per well.
  - **Note:** Make fresh daily.

- Plate 4 = Wash 3
    - Add 200µl Wash, Custom per well.

- Plate 5 = Bind 2
    - Add 200µl Binding Solution 2 per well.

- Plate 6 = Wash 2 DNase
    - Add 190µl RNA DNase Wash, Custom and 10µl resuspended DNase I per well.
    - **Note:** To ensure that the DNase I is active during the DNase step, make certain to dispense the DNase Wash and DNase I mixture to Plate 6 after prompt from method.

- Plate 7 = Wash 1
    - Add 900µl Wash, Custom per well.

- Plate 8 = Lysis and Bind Plate
    - Add 200µl of Lysis Buffer per well.
    - Add 25µl of Proteinase K per well.
    - **Note:** The Lysis Buffer and Proteinase K can be combined into one tube and dispensed into the plate. Keep in mind that, once the Lysis Buffer and Proteinase K are combined, the Proteinase K will start to lose activity after 30 minutes.

Protocol:

Total Run Time = 1:30

1. Homogenize sample by one of the following methods:
    a. Homogenize by liquid nitrogen grinding, and then add 600µl of chilled 1-Thioglycerol/Homogenization Solution to 20–50mg of the plant sample.
    b. Add 600µl of chilled 1-Thioglycerol/Homogenization Solution bead beating.
2. Using a large-bore pipet tip, add 400µl of homogenate to a 96 well plate or 1.5ml tube containing 200µl of Lysis Buffer.
3. Allow sample to incubate at room temperature for 10 minutes.
4. Centrifuge plate or tubes at max speed for 2 minutes.
5. Add 400µl of supernatant, 333µl of Binding Buffer III, and 35µl of ReliaPrep™ Resin to each well of Plate 8.
6. Start method.
7. Following lysis, when prompted manually, add 190µl of RNA DNase Wash and 10µl of resuspended DNase I to each well of Plate 6.
8. Continue method.

Results:

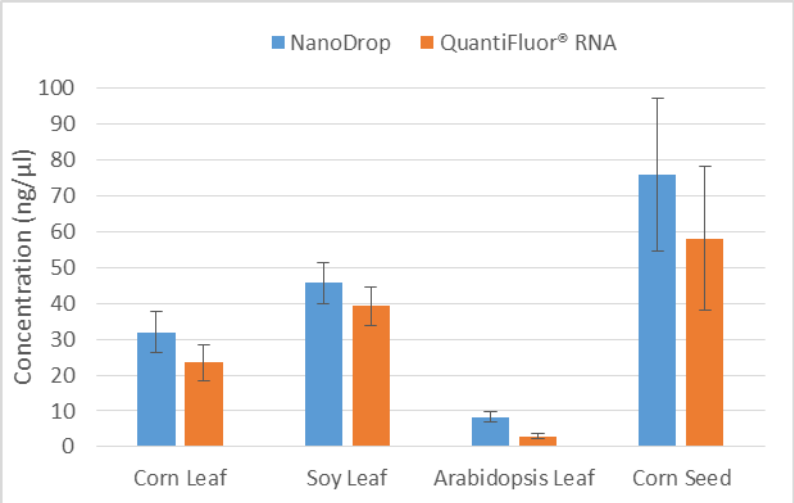

Figure 1. RNA concentration from plant tissue isolated with the Maxwell® HT simplyRNA kit on the KingFisher™ Flex. RNA concentration was determined using the NanoDrop®-1000 and QuantiFluor® RNA System on the Quantus™ Fluorometer (N=8).

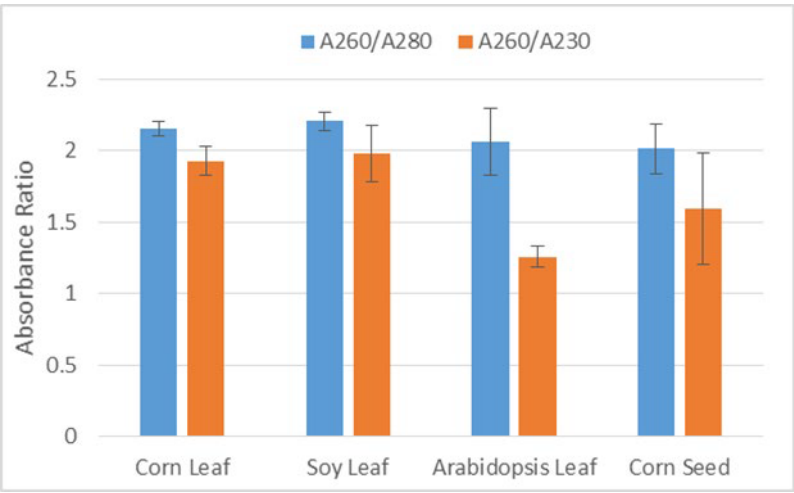

Figure 2. RNA purity from plant tissue with the Maxwell® HT simplyRNA kit on the KingFisher™ Flex. RNA purity was determined using the NanoDrop®-1000 (N=8).