Promega

Product Application

RNA Purification from Oil Palm Leaf using the Maxwell® RSC System

Isolate high-quality, amplifiable RNA from oil palm leaf tissue using the Maxwell® RSC System.

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

Analyses: NanoDrop, QuantiFluor® quantitation, TapeStation, 1- Step RT-qPCR

Sample Type(s): Oil Palm, *Elaeis guineensis*

Input: 20mg, 40mg, 60mg, 80mg and 100mg of leaf tissue

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

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC Plant RNA Kit (Cat.# AS1500)
- mortar and pestle
- liquid nitrogen
- tissue homogenizer (e.g., Tissue-Tearor™ Homogenizer)
- microcentrifuge

Protocol:

1. Grind tissue and sample material in liquid nitrogen using a mortar and pestle.
2. Add up to 100mg of ground sample to a 2ml tube.
3. Add 600µl of chilled 1-Thioglycerol/Homogenization Solution to the tube.
4. Homogenize samples with a small tissue homogenizer until homogenized, and then place on ice.
5. With a wide-bore pipet, transfer 400µl of homogenate to a microcentrifuge tube.
6. Add 200µl of Lysis Buffer to the homogenate. Vortex vigorously for 15 seconds to mix.
7. Incubate at room temperature for 10 minutes.
8. Centrifuge for 2 minutes at 16,000 × *g*.
9. Place the cartridge into the Maxwell® RSC cartridge rack and remove the seal.
10. Transfer the clear supernatant to Well #1 of the Maxwell® cartridge.
11. Add 5µl of DNase to Well #4.
12. Place the one of the supplied elution tubes into the sample rack, and add 50µl of the supplied Nuclease-Free Water for each sample.
13. Place the plunger into Well #8.
14. Select Maxwell® RSC Plant RNA Kit on the Maxwell® and run the method.

Results:

RNA was purified from oil palm leaves with the Maxwell® RSC Plant RNA Kit using 20mg, 40mg, 60mg, 80mg and 100mg of input.

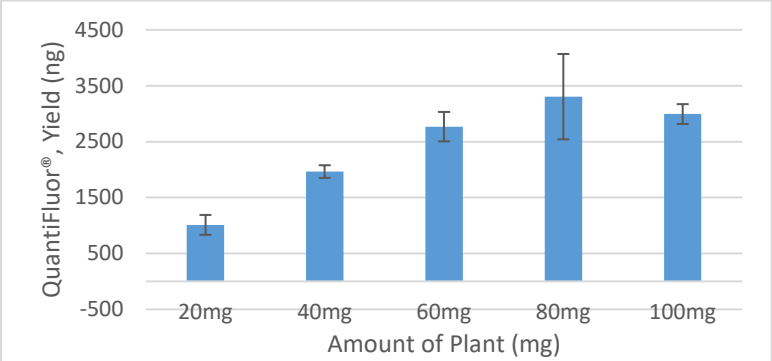

Figure 1. RNA yield purified from oil palm leaf and measured by QuantiFluor® RNA System. Examples of RNA yield based on volumes and QuantiFluor® data.

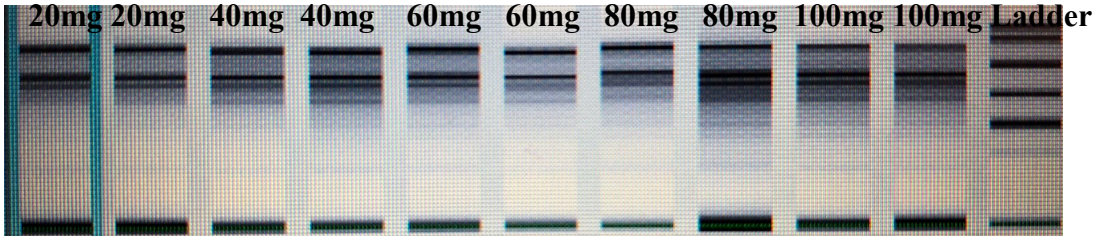

Figure 2. RNA integrity for purified oil palm RNA. Examples of high-quality RNA purified from the indicated amount and analyzed on Agilent TapeStation using RNA ScreenTape.

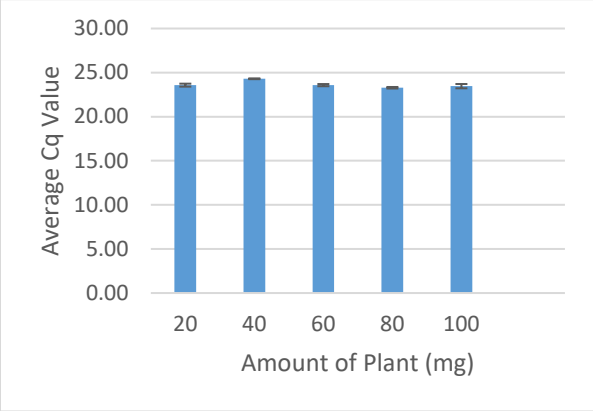

Figure 3. 1-Step RT-qPCR amplification of purified oil palm RNA. GoTaq® 1-Step RT-qPCR amplification with Actin primers using 1µl of RNA eluate per reaction.

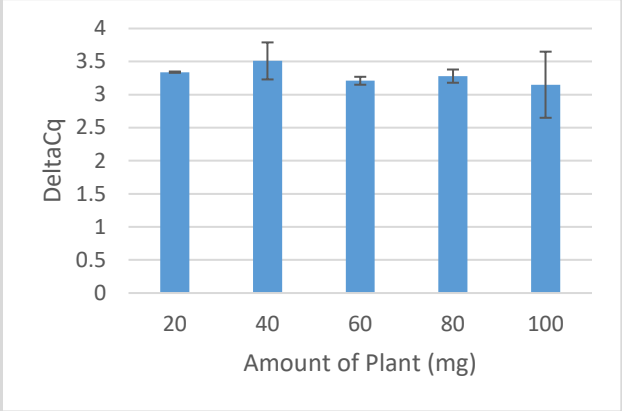

Figure 4. 1-Step RT-qPCR amplification inhibition measured by ΔCq values for RNA purified from oil palm. Samples were amplified undiluted, and diluted 1/10. A ΔCq of ~3.3 indicates no qPCR inhibition.