Promega

Product Application

Automated Purification of Fungal RNA

Purify RNA from fungal fruiting bodies and mycelium using the Maxwell® RSC Plant RNA Kit on a Maxwell® RSC Instrument.

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

Analyses:

- UV absorbance
- RT-qPCR
- TapeStation Analysis

Sample Type(s): Fungal fruiting body (*Hericium*, *Ganoderma*)
Fungal mycelium (*Ganoderma*)

Input: 20-50mg

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**

Materials Required:

- Maxwell® RSC Plant RNA Kit (Cat.# AS1500)
- Maxwell® RSC Instrument or Maxwell® RSC 48 Instrument
- Liquid nitrogen, mortar, and pestle *OR*
- Bead beating supplies
  - Lysing Matrix A – MP Biomedicals, Cat.# 6910050
  - Digital Vortex Genie II – Scientific Industries or similar
  - Horizontal Vortex Adaptor for 1.5-2.0ml tubes – Qiagen, Cat.# 13000-V1 or similar

Protocol:

1. Prepare 1-Thioglycerol/Homogenization Solution and DNase I according to the technical manual and chill on ice.
2. If using a mortar and pestle for sample disruption
   a. Transfer tissue to a mortar pre-cooled with liquid nitrogen.
   b. Grind to a fine powder using a pre-cooled pestle.
   c. Transfer 20-50mg of ground tissue to a pre-cooled 1.5ml tube and keep frozen on dry ice until ready to process.
   d. Add 400µl of prepared 1-Thioglycerol/Homogenization Solution and vortex for 30 seconds.
3. If using bead beating for sample disruption
   a. Transfer 20-50mg of tissue to a pre-cooled Lysing Matrix A tube and keep frozen on dry ice until ready to process.
   b. Add 400µl of prepared 1-Thioglycerol/Homogenization Solution.

   c. Secure sample tubes on a digital vortex outfitted with a horizontal tube adaptor. Vortexes may require balancing of tubes and/or limited tube numbers to maintain vortex speed.
   d. Vortex for 10 minutes at 2600-3000rpm.
4. Add 200µl of Lysis Buffer and vortex for 15 seconds.
5. Incubate at room temperature for 10 minutes.
6. During incubation, prepare RSC cartridges as described in the Maxwell® RSC Plant RNA Kit Technical Manual.
   a. Add 5µl of prepared DNase I to Well #4.
   b. Place a plunger in Well #8.
   c. Add 50µl of Nuclease-Free Water to each elution tube.
7. After incubation, centrifuge samples for 2 minutes at maximum speed.
8. Transfer the entire supernatant to Well #1 of the reagent cartridge. Avoid pipetting any solid material.
9. Place the prepared cartridges in the Maxwell® RSC Instrument and run the Maxwell® RSC Plant RNA Kit method.

Results:

RNA was purified from fungal fruiting bodies or mycelia using the Maxwell® RSC Plant RNA Kit using the protocol above. This protocol can purify high integrity RNA that is suitable for RT-qPCR.

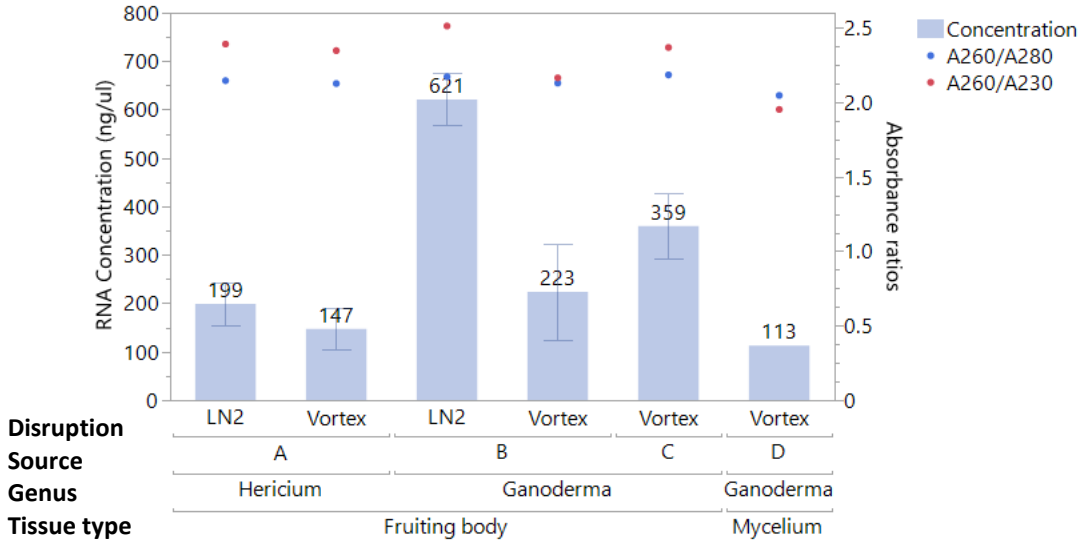

Figure 1. Concentration and absorbance ratios of RNA purified from *Hericium* fruiting body, or *Ganoderma* fruiting body or mycelium, using the Maxwell® RSC Plant RNA Kit. RNA was purified from fungal fruiting bodies (A,B,C) or mycelium (D) using either disruption by grinding under liquid nitrogen (LN2) or vortexing in Lysing Matrix A tubes as described in the protocol above. RNA concentration and A260/A280 and A260/A230 absorbance ratios were measured using a NanoDrop™ ONE Spectrophotometer. Mean values ± stdev of n=3; n=1 for mycelium.

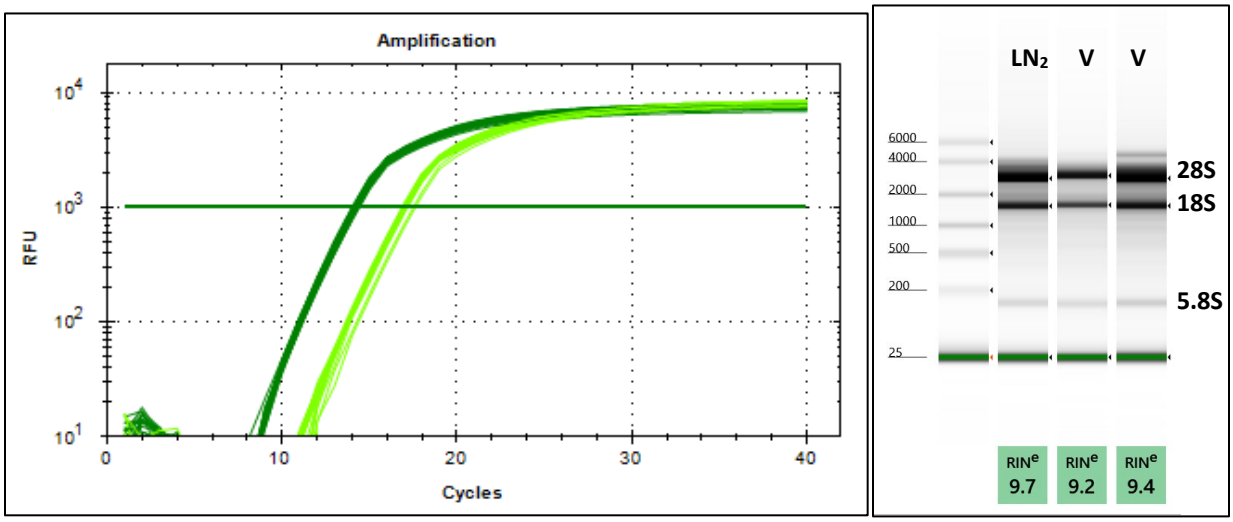

Figure 2. *Hericium* RNA amplification and integrity. (Left) *Hericium* RNA eluates shown in Fig.1 were serially diluted to 20ng/µl (dark green) and 2ng/µl (light green) in Nuclease-Free Water (Cat.# P119E) and 2µl used for amplification. The fungal ITS region was amplified in duplicate 20µl reactions with GoTaq® 1-Step RT-qPCR System (Cat.# A6020) on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Cat.# 1855195). Amplification curves are shown on a semi-log plot. (Right) RNA integrity was evaluated using RNA ScreenTape (Agilent, Cat.# 5067-5576) on a 4200 TapeStation (Agilent) for a subset of *Hericium* disrupted by grinding under liquid nitrogen (LN_2) or by vortexing in Lysing Matrix A tubes (V). RNA Integrity Number equivalents (RIN^e) are indicated for each sample.