Promega

Product Application

Automated Purification of RNA from Mold Spores

Purify RNA from Aspergillus mold spores 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): Mold spores (*Aspergillus*)

Input: 20-50mg

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
- Optional: ReliaPrep™ RNA Clean-Up and Concentration System – Cat.# Z1071

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. 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 for several minutes 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.
10. *Optional*: RNA eluates purified from some mold spores may be colored and/or contain amplification inhibitors. Both can be reduced using the ReliaPrep™ RNA Clean-Up and Concentration System following purification on the Maxwell® RSC Instrument. Alternatively, sample dilution may reduce amplification inhibition.

Results:

RNA was purified from *Aspergillus* spores using the Maxwell® RSC Plant RNA Kit. RNA eluates were tinted, and the most heavily colored eluates (Sample 2, LN2) were further purified using the optional ReliaPrep™ RNA Clean-Up and Concentration System before amplification. All RNAs were of high integrity and could be used as templates for RT-qPCR when diluted.

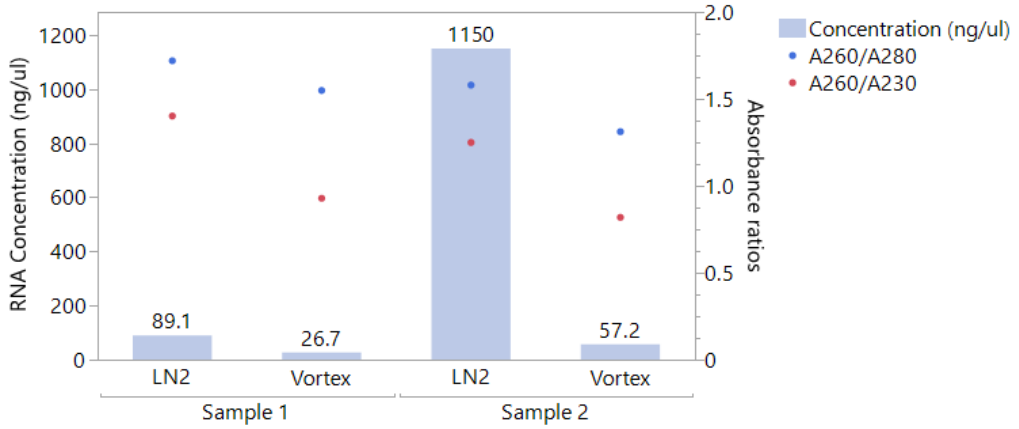

Figure 1. Concentration and absorbance ratios of RNA purified from *Aspergillus* spores using the Maxwell® RSC Plant RNA Kit. RNA was purified from two distinct samples of *Aspergillus* spores using either disruption by grinding under liquid nitrogen (LN2) or vortexing for 10 minutes in Lysing Matrix 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 are shown for samples ground under liquid nitrogen (n=2); n=1 for samples disrupted by vortexing.

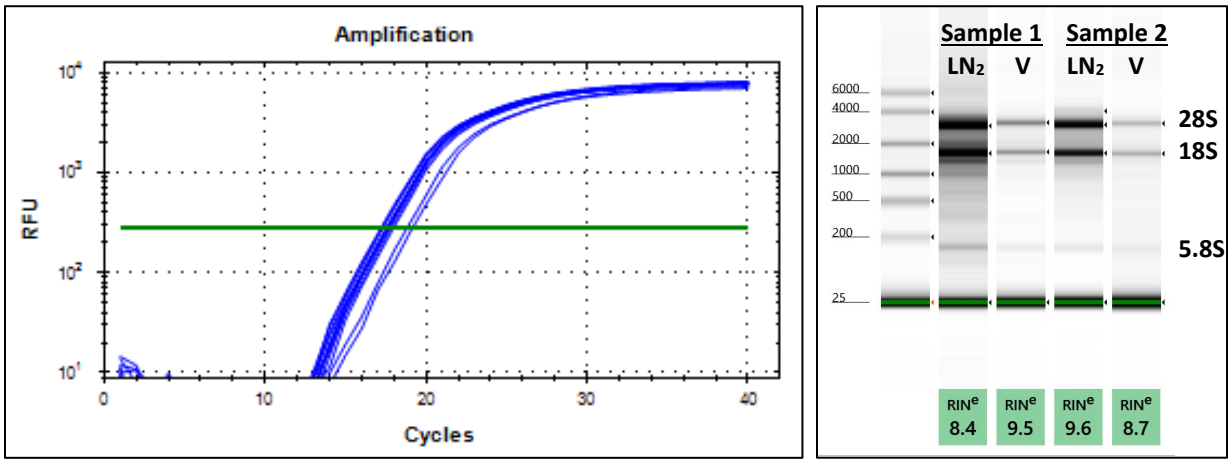

Figure 2. RNA amplification and integrity. (Left) RNA eluates shown in Figure 1 were diluted to 2ng/µl in Nuclease-Free Water (Cat.# P119E) and 2µl was used for amplification. The fungal ITS region was amplified in duplicate 20µl reactions with GoTaq® 1-Step RT-qPCR System (Cat.# A6020) on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad, Cat.# 1855195). Amplification curves are shown on a semi-log plot. (Right) RNA integrity was evaluated for a subset of eluates disrupted by grinding under liquid nitrogen (LN_2) or by vortexing in Lysing Matrix A tubes (V) using RNA ScreenTape (Agilent, Cat.# 5067-5576) on a 4200 TapeStation (Agilent). RNA Integrity Number equivalents (RIN^e) are indicated for each sample, with values of 8.0-10.0 typically indicating high integrity RNA. Sample 1, LN_2 was diluted 1/5 due to sample concentration; All other samples were electrophoresed without dilution.