Promega

Product Application

Automated Bacterial RNA Extraction

RNA extraction from Gram-positive and Gram-negative bacteria using the Maxwell® RSC miRNA Tissue Kit.

Kit: Maxwell® RSC miRNA Tissue Kit (Cat.# AS1460)

Analyses: UV absorbance, dye-based quantitation, TapeStation

Sample Type(s): Bacterial culture

Input: Up to 5 x 10^8 cells

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 miRNA Tissue Kit (Cat.# AS1460)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Lysozyme (10µg/µl)
- 20X TE Buffer (pH 7.5) (Cat.# A2651)
- Heat block

Protocol:

Before starting, prepare the 1-Thioglycerol/Homogenization and DNase I solutions as indicated in Technical Manual #TM441.

1. Aliquot 500µl of bacterial culture in 1.5ml microtubes, pellet at 8,000 x *g* for 5 minutes and remove the supernatant.

   Gram-negative bacteria

   a) Resuspend pellets in 200µl of 1-Thioglycerol/Homogenization Solution by vortexing or pipetting and continue to step 2.

   Gram-positive bacteria

   a) Add 400µl of 1X TE Buffer and 100µl of lysozyme (10 µg/µl) and incubate at 37 °C for 30 minutes. Centrifuge tube at 2,000 x *g* for 5 minutes and discard the supernatant to obtain the lysed cells.

   b) Resuspend pellets in 200µl of 1-Thioglycerol/Homogenization Solution by vortexing or pipetting and continue to step 2.

2. Add 200µl of Lysis Buffer and 200µl of Lytic Enhancer, vortex vigorously for 20 seconds to mix.
3. Add 30µl of Proteinase K and incubate at 37 °C for 15 minutes.
4. During the incubation prepare cartridges as indicated in Technical Manual #TM441.
5. Add lysate to well #1 and start the miRNA Tissue method on the Maxwell® RSC Instrument.

Results:

RNA from Gram-positive and Gram-negative bacteria was successfully purified using Maxwell® RSC miRNA Tissue Kit, adding a lysozyme digestion step for Gram-positives.

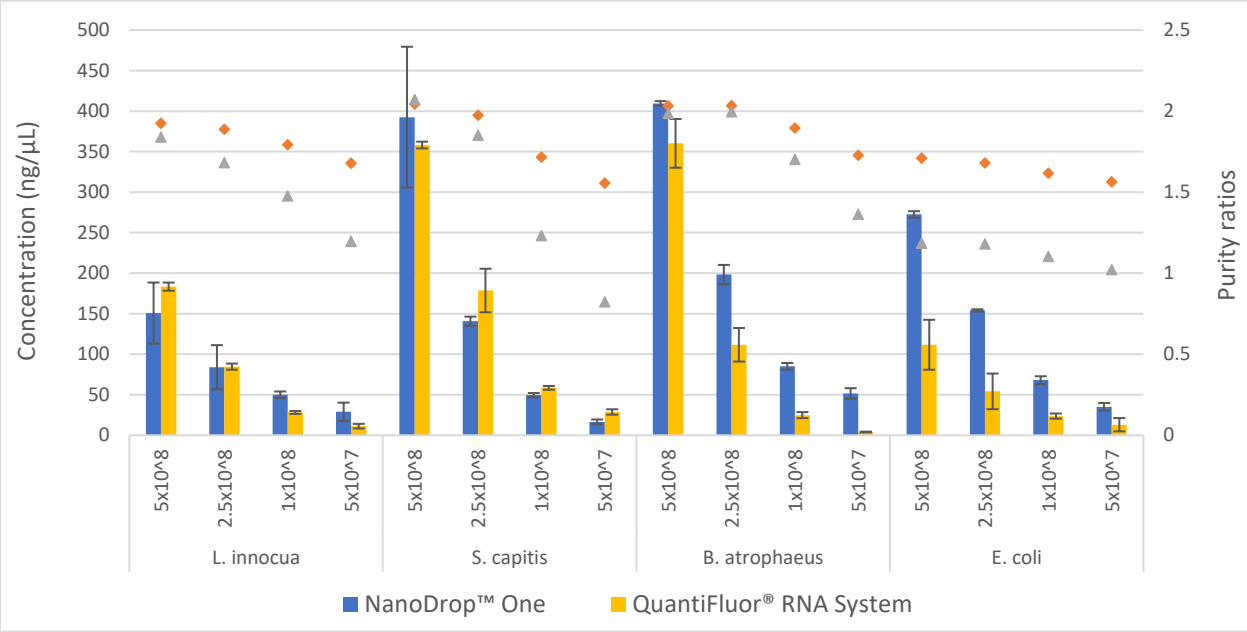

Figure 1. RNA concentrations and purity ratios obtained using Maxwell® RSC miRNA Tissue Kit. RNA was purified from 5x10^8, 2.5x10^8, 1x10^8, 5x10^7 cells of *L. innocua*, *S. capitis*, *B. atrophaeus* and *E. coli*. RNA concentration and purity ratios were measured by absorbance using a NanoDrop™ One Spectrophotometer. RNA concentration was also measured using QuantiFluor® RNA System (Cat.# E3310) on a GloMax® Discover Microplate Reader (Cat.# GM3000) Data represents mean ± standard deviation of N=3. Extraction from *E. coli* was performed without the lysozyme digestion.

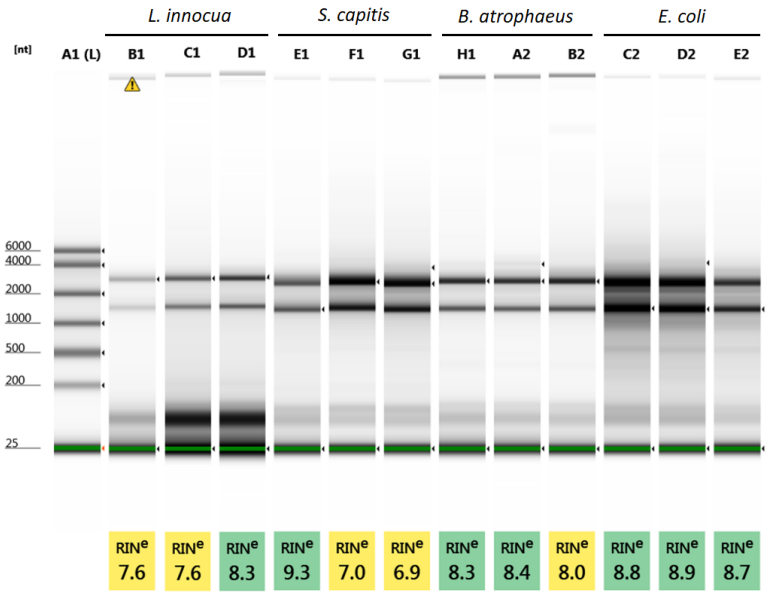

Figure 2. TapeStation 4200 System results for RNA samples purified using Maxwell® RSC miRNA Tissue Kit. Tapestation electrophoresis of DNA purified in triplicate from 5x10^8, cells of *L. innocua*, *S. capitis*, *B. atrophaeus* and *E. coli.*