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Application News

MultiNA™ Microchip Electrophoresis System

Quality Assessment of Total RNA Using MultiNA Automated Analysis

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User Benefits

- MultiNA automates almost the entire electrophoresis workflow.
- MultiNA automatically determines the ratio of 28S rRNA to 18S rRNA (28S/18S) and displays it in a report.
- MultiNA outputs numeral results that allow an objective evaluation of the total RNA quality.

■ Introduction

The extraction of total RNA from biological tissue samples is an important process and the starting point for a wide range of analytical techniques, such as northern blotting, cDNA library construction, cloning, sequencing (next-generation sequencing and Sanger sequencing), and PCR (real-time PCR and qualitative PCR). Sample collection, preparation, and storage conditions also have a major impact on the quality and yield of a total RNA sample.

Following extraction, the quality of total RNA in the sample is assessed by ultraviolet (UV) spectroscopy and electrophoresis (denatured agarose gel electrophoresis).

The ratio of sample absorbance at 260 to 280 nm is used to test RNA sample purity, where a ratio (260/280 nm) of 2.0 is generally considered the benchmark for pure RNA. A ratio below 2.0 raises concerns over adulteration of the total RNA sample by proteins or reagents used in sample preparation. RNA is also considered more susceptible to degradation than DNA. Electrophoresis can be used to assess the degree of degradation of total RNA samples based on the above-mentioned ratio of 28S rRNA to 18S rRNA. When analyzed by electrophoresis, a sample free from impurities displays a clear 28S rRNA peak and 18S rRNA peak. Since the size ratio of 28S rRNA to 18S rRNA *in vivo* is 2:1, the 28S and 18S peaks in a high-quality RNA sample are detected in the ratio of 2:1 after electrophoresis analysis.

When electrophoresis analysis of RNA is performed on denatured agarose gel, every step of the process is labor intensive, from gel preparation and sample application to performing electrophoresis, signal detection, and clean up.

This article describes a quality assessment of a total RNA sample performed using a Shimadzu microchip electrophoresis system (Fig. 1).

Fig. 1 MCE-202 MultiNA™ Multichip Electrophoresis System

Table 1 Total RNA Analytical Conditions

System:	MCE-202 MultiNA
Project:	Total RNA
Kit:	RNA kit (Shimadzu)
Fluorescent Dye:	SYBR Green II (Thermo)
Ladder:	RNA 6000 Ladder (Thermo)

■ Samples and Analytical Conditions

Sample Preparation

Rat Kidney Total RNA (Clontech) was used as the RNA sample. The RNA sample was prepared by diluting an undiluted RNA solution (1 µg/µL) to 50 ng/µL with TE buffer. The change in RNA over time was investigated by testing the RNA sample solution after storing for zero weeks (the day of preparation), 1 one week (at room temperature), and two weeks (at room temperature).

RNA Analysis on MultiNA System

Sample analysis was performed on the MultiNA system in premix mode (Table 1). The RNA samples were combined with a marker from an RNA kit in the ratio of 1:1. The ladder solution was prepared by diluting the stock ladder solution (RNA 6000 Ladder) six-fold and combining it with the same marker from the RNA kit in the ratio of 1:1. The ladder solution and RNA sample (each contained the marker) were then subjected to thermal treatment at 65 °C for five minutes then 4 °C for five minutes. After thermal treatment, each solution was loaded in the MultiNA electrophoresis system together with the separation buffer for analysis (Fig. 2).

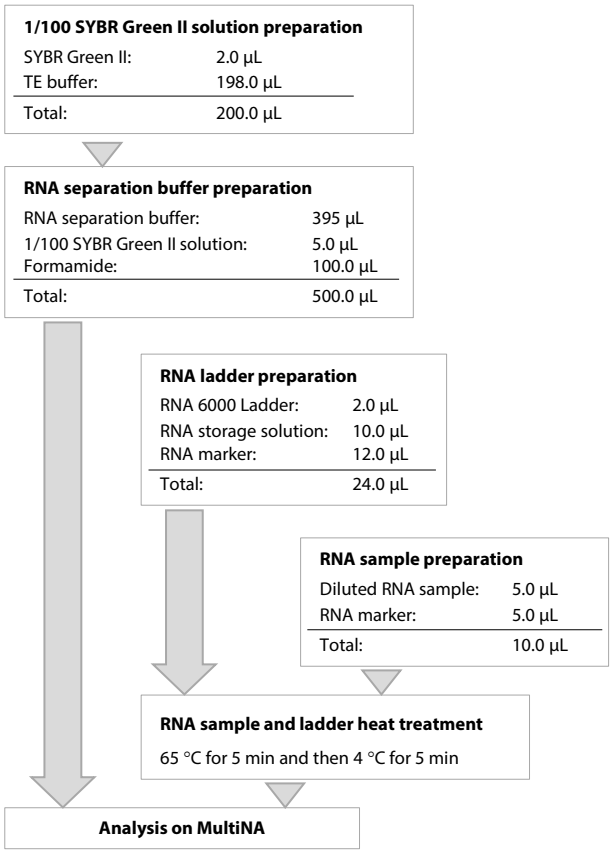

Fig. 2 Procedure for Analysis by MultiNA (for 6 Samples)

■ Results

Samples of Rat Kidney Total RNA (50 µg/µL) stored at room temperature for zero, one, and two weeks were analyzed by the MultiNA electrophoresis system. Fig. 3 shows the resulting gel image, and Fig. 4 shows the corresponding electropherogram. All of the total RNA samples produced two major peaks (bands) in addition to the low molecular weight marker (LM) (Figs. 3 and 4). These peaks were identified as 18S rRNA (the white triangle) and 28S rRNA (the shaded triangle) based on their position relative to the RNA 6000 Ladder. The peaks in the electropherogram were also identified automatically by the MultiNA system. Each peak was labeled with the results, and both the 18S rRNA (the white arrow) and 28S rRNA (the shaded arrow) were correctly identified by the MultiNA system.

MultiNA was also able to automatically analyze the data, calculate the ratio of 28S rRNA to 18S rRNA (28S/18S), and display it in an RNA report it created. Using this feature, the ratio of 28S rRNA to 18S rRNA in samples of Rat Kidney Total RNA (50 ng/µL) stored for zero, one, and two weeks at room temperature were calculated as 2.70, 1.84, and 1.63, respectively. Given that the ideal ratio of 28S rRNA to 18S rRNA (28S/18S) is 2:1 (2.00), this analysis shows the sample at zero weeks (the day of preparation) was of sufficiently high quality. The 28S/18S ratio of the samples stored for one and two weeks were both below this ideal level (1.84 and 1.63, respectively), showing that the ratio fell with each day of storage.

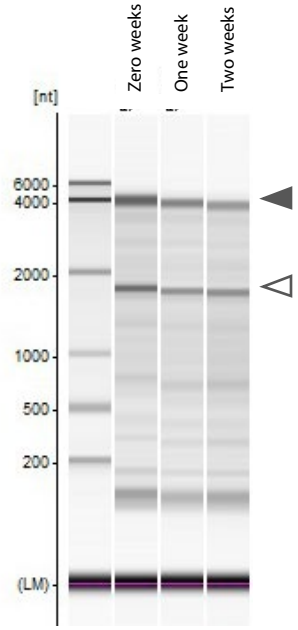

Fig. 3 MultiNA Gel Image Showing Effect of Storage Time on Rat Kidney Total RNA (50 µg/µL)
(Zero, One, and Two Weeks at Room Temperature)

■ Conclusion

The MultiNA electrophoresis system was used to analyze a total RNA sample and output the ratio of 28S rRNA to 18S rRNA in the sample as a numerical value. This numerical value was then used to assess the quality of the total RNA sample over time. Compared to using denatured acrylamide gel, electrophoresis on MultiNA for quality assessments of total RNA not only provides the labor-saving benefits of fully automated analysis but also allows for an objective assessment of RNA quality based on numerical data. These features are even more useful in applications that must process large numbers of samples and require high analytical throughput.

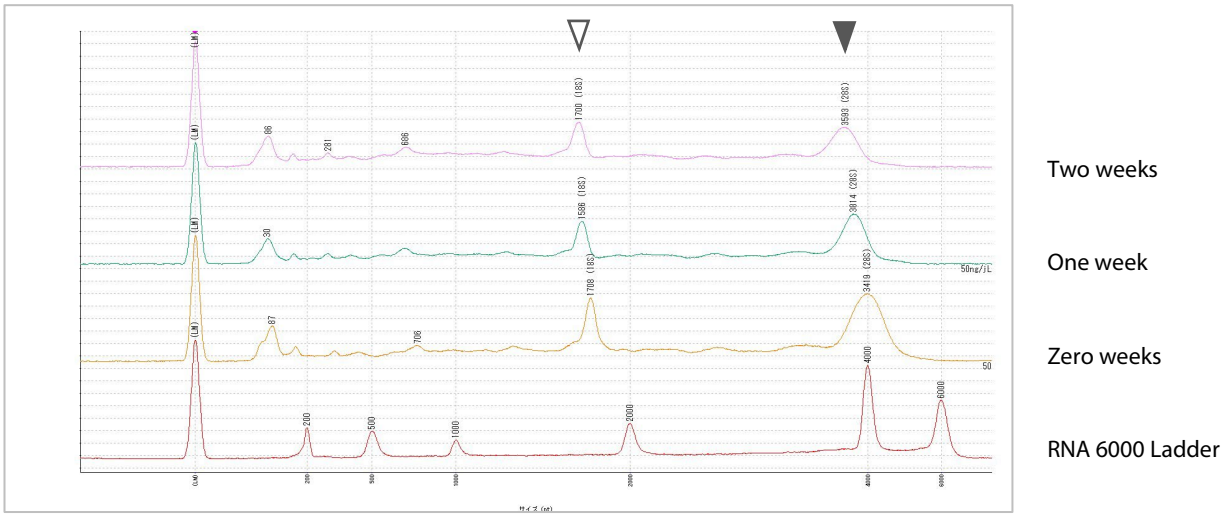

Fig. 4 MultiNA Electropherogram Showing Effect of Storage Time
(Zero, One, and Two Weeks at Room Temperature) on Rat Kidney Total RNA (50 µg/µL)

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