Preservation of RNA in Soil, Plant and Stool Samples Using Norgen's RNA Preserve

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INTRODUCTION

In research, RNA is extensively being studied by RNA-Seq and sRNA-Seq to better comprehend the role RNA has in protein synthesis and gene expression[(1,2)]. It is known that RNA is inherently unstable in samples outside their physiological environment, and is sensitive to temperature and RNAses, which are detrimental to its integrity. This is a common problem for researchers, who require good quality RNA to perform analysis, especially when samples must be sent out for service. To overcome this problem, costly methods such as shipping on dry ice or even refrigerated shipping have been implemented to conserve the quality of RNA. These methods may be accessible to some companies, but in many instances the cold storage method is not feasible due to limited equipment or funds. Alternatively, there are chemical preservatives on the market that conserve the integrity of RNA being stored at room temperature. This method of preservation both protects the integrity of RNA, and saves costs by eliminating the need for cold shipments. This application note demonstrates the effectiveness of Norgen's RNA Preserve for stabilizing samples such as soil, plant, and stool.

MATERIALS AND METHODS

Sample Collection

Plant

Grape leaf disks 0.7cm in diameter were collected from the same leaf and stored in tubes already containing beads for efficient processing of samples. 6 grape leaf disks were used for each of the three conditions tested: storage at room temperature with 1mL of Norgen's RNA Preserve, storage at room temperature without preservative and storage at -70°C. Samples were left for two days at each storage condition, followed by RNA extraction.

Soil

For soil, a 1mL *E. coli* culture was first spun down and the supernatant was removed. Next, 200 mg of soil was added to the *E. coli* pellet and then mixed with 400 µL of preservative. Three different preservatives were tested: Norgen's RNA Preserve and two competitors. The preserved soil samples were all stored at room temperature, and RNA was isolated at days 7 and 14.

Stool

Stool was collected by one individual, and 200 mg of stool was suspended in 2 mL of Norgen's RNA Preserve. The stool sample was stored at room temperature and samples were processed for RNA extraction at time points 7, 10, 14, and 21 days.

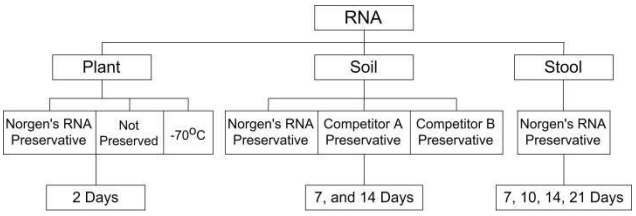

Figure 1. Summary of the experiments to study Norgen's RNA Preserve with plant, soil, and stool samples. The samples were kept at room temperature with the exception of the -70°C storage condition for the plant sample.

RNA Extraction

Plant

To extract RNA from the plant samples, Norgen's RNA preservative was first aspirated from the bead tube. With the preservative removed, the three conditions containing 6 grape leaf disks each were then homogenized. The experiment utilized the Bead Ruptor Elite from Omni International at 5 M/S for 40 seconds. The RNA was further processed following Norgen's Plant/Fungi Total RNA Purification Kit (Cat# 25800). The quality and quantity of RNA was measured using a Nano RNA chip, and analyzed by Agilent Technologies 2100 expert v. B.02.08.SI468.

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Soil

To isolate RNA from soil, the preserved sample was first spun down at 6,000 rpm for 3 minutes. Next, Norgen's RNA Preserve was carefully removed and the soil was resuspended in 0.75 mL of Lysis Buffer. The suspended soil was then transferred into a bead tube. The soil was homogenized using the Bead Ruptor Elite at 4 M/S for 30 seconds. The isolation was continued using Norgen's Soil Total RNA Purification Kit (Cat# 27750), and the RNA quality and quantity was visualized by gel electrophoresis.

Stool

Stool RNA was isolated from 0.2 mL of preserved stool utilizing Norgen's Stool Total RNA Purification Kit (Cat# 49500). The Bead Ruptor Elite from Omni International was used at 5 M/S for 30 seconds to homogenize the preserved stool with the 0.8 mL of Lysis Buffer. The quality and relative amounts of RNA isolated from stool was visualized using gel electrophoresis.

Gel Electrophoresis

For visual analysis of the RNA from both soil and stool, 7.5 µL of their 50 µL and 75 µL elution respectively were loaded onto a 1.4% 1x MOPS gel. The gels were run for 20 minutes at 170V, and the image taken by an AlphaImager™ IS-2200 (Alpha Innotech).

RESULTS AND DISCUSSION

Plant

The RNA in the grape leaf disks stored at room temperature in Norgen's RNA Preserve compared to the leaf disks stored at -70°C had similar RNA degradation profiles. The gel photo attained from the 2100 expert software **(Figure 1A)** shows that the RNA is less abundant in the samples stored at room temperature without any preservative. Specifically, the lower lying RNA band outlined in the red box shows the difference in the RNA degradation between the methods of preservation after the two days. In addition, the electropherogram **(Figure 1B)** better shows the RNA degradation profiles of the three preservative methods. The blue arrow from the Not preserved panel in Figure 1B indicates a peak that is missing but is still present in the samples preserved in Norgen's RNA Preserve and those stored at -70°C.

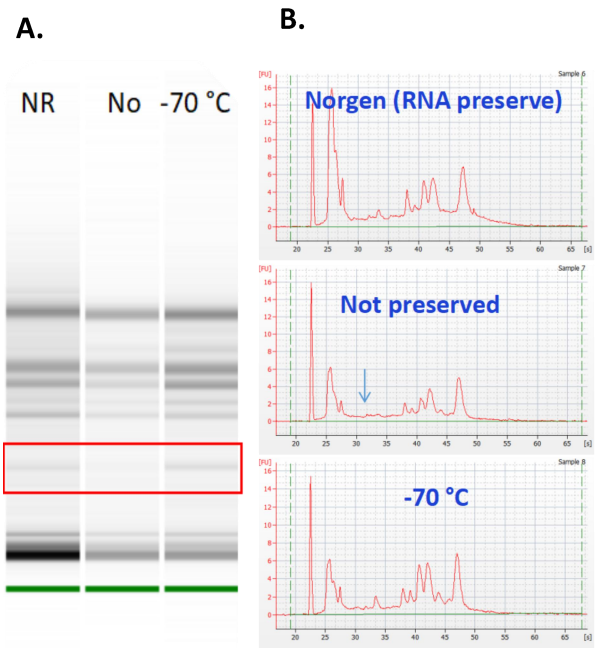

Figure 1A. The gel photo attained from the 2100 expert software shows the RNA present in the three preservation methods. NR: Norgen's RNA Preserve, No: Not preserved. **Figure 1B.** The electropherogram analyzed by the 2100 expert software shows the RNA degradation profiles of the three preservation methods.

Soil

The RNA isolated from preserved soil kept at room temperature on day 7 **(Figure 2A)** and day 14 **(Figure 2B)** further supports the effectiveness of Norgen's RNA Preserve. In caparison between day 7 and day 14, Norgen's RNA Preserve maintains the integrity of the RNA, while competitor A and B show signs of degradation after 14 days.

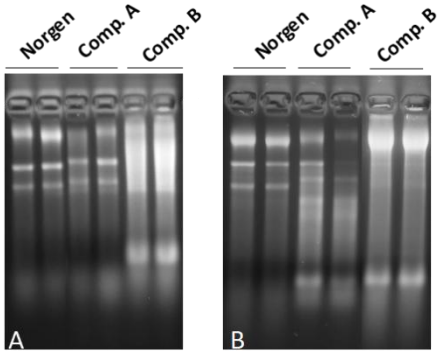

Figure 2. 7.5 µL of a total of 50 µL of isolated RNA from preserved soil on days 7 **(A)** and days 14 **(B)** was run an 1.4% 1x MOPS gel. As seen in the figure, Norgen's RNA preservative best maintained the integrity of the RNA over the period of storage at room temperature.

Stool

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In this experiment, stool was kept at room temperature in Norgen's RNA Preserve for 21 days. The RNA was isolated on days 7, 10, 14, and 21 **(Figure 3)** and as seen in the figure, the different days all have the same RNA degradation profile indicating successful preservation of the RNA over the 21 day period.

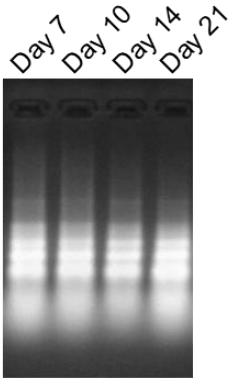

Figure 3. 7.5 µL of a total elution of 75 µL of the RNA preserved in stool was run on a 1.4% 1X MOPS gel. The figure shows that Norgen's RNA Preserve can maintain the quality of RNA in stool for up to 21 days.

CONCLUSIONS

This study testing Norgen's RNA Preserve with grape leaf disks, soil, and stool proves its effectiveness in terms of maintaining RNA integrity over periods of storage at room temperature. Table 1 summarize the recommended sample input sizes and storage conditions.

Table 1. Summary of recommended sample input sizes and storage conditions for RNA preservation with Norgen's RNA Preserve.

Sample type	Input Sample size	Amount of RNA Preserve to add	Storage condition		
			Room Temperature*	4 °C	- 20 °C & - 80 °C
Plant	0.7 cm leaf disk (up to 6 disks) or similar volume	5 volume of the fresh sample	2-3 days	Up to 1 month	For archival samples
Stool	500 mg-1g	1-2 mL (1:2 ratio)	1 week		
Soil	200 mg	400 µL (1:2 ratio)	2 week		

REFERENCES

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Related Products	Product #
Stool Total RNA Purification Kit	49500
Stool Total RNA Purification Kit Dx	Dx49500
Stool Nucleic Acid Isolation Kit	45600
Plant / Fungi Total RNA Purification Kit	25800, 31350, 25850
Soil Total RNA Purification Kit	27750

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