Stabilization of large volume RNA samples dried in 96-well deep well plates with RNAstable® LD

INTRODUCTION

RNAstable® LD (Biomatrica Catalog #53201-013) is a novel, liquid, RNA preservation product that allows for purified RNA to be dried and stored in any tube or plate format. RNAstable® LD protects RNA samples from degradation during storage and shipment at ambient temperatures in a dry environment. Biomatrica's standard protocol allows for drying RNA samples of up to 100 µL. The data presented below shows RNA integrity of large volume samples dried and stored at ambient temperature, with or without RNAstable® LD.

MATERIALS AND METHODS

Pre-processing

Total RNA was isolated from human whole blood samples using MagNA Pure Compact RNA Isolation Kit (Roche Catalog #04802993001) following the manufacturer's instructions. The isolated total RNA concentration was 84.1 ng/µL based on NanoDrop 2000 UV-Vis Spectrophotometer reading. Total RNA was stored at -80°C in 1 mL aliquots prior to use.

Sample preparation

RNA was thawed on ice and diluted to a final concentration of 25 ng/µL in nuclease-free water. Approximately 250 ng and 500 ng of RNA were immediately visualized on 1% agarose gel to verify no degradation of the RNA during the -80°C storage. Three aliquots of 50 µL each were stored at -80°C as positive controls. Triplicate aliquots of 200 µL RNA stock were added to the wells of a 0.75 mL Micronic U-bottom 96-well storage tube (Micronic Catalog #MPW75128BCS) with or without 40 µL of RNAstable® LD.

Sample dry down & storage

Samples in the 96-well plate were dried using a vacuum concentrator. RNAstable® stabilized RNA samples took 4 hours of drying time, whereas unprotected RNA samples required additional drying time. The 96-well plate was then sealed with AeraSeal sealing films, and stored overnight at room temperature in a sealed moisture-barrier silver foil bag with desiccant packs.

Sample rehydration

RNA was rehydrated with 50 µL of nuclease-free water and used directly in agarose gel and Bioanalyzer analyses.

Agarose gel analysis

500 ng of rehydrated RNA was visualized on a 1.2% agarose gel containing ethidium bromide.

Bioanalyzer analysis

1 µL of rehydrated RNA was analyzed with the Agilent RNA 6000 Nano kit using the Agilent 2100 Bionanalyzer following manufacturer's instructions. Gel image, RIN score, and electropherograms were obtained for analysis and confirmation. The RIN, or RNA Integrity Number (a score of 1-10 algorithmically calculated by the system using the 28S/18S ratio, the region before the peaks, signal areas and intensities), was used to determine the RNA quality. A RIN of 10 corresponds to most intact RNA while a RIN of 1 corresponds to completely degraded RNA.

RESULTS

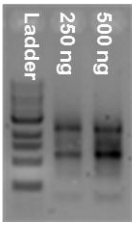

Figure 1: Initial verification of RNA quality prior to use. 250 ng and 500 ng of purified RNA were visualized on a 1% agarose gel to verify integrity of the sample before stabilizing with RNAstable®.

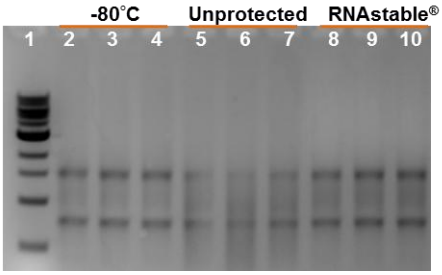

Figure 2: Gel electrophoresis analysis of the quality of rehydrated stabilized in RNAstable® overnight. 500 ng of RNA samples were visualized on a 1.2% agarose gel. Lanes 2 to 4 contain samples thawed following overnight storage at -80°C. Unprotected samples (lanes 5 to 7) or samples stabilized with RNAstable® (lanes 8 to 10) were analyzed following dehydration, overnight storage and rehydration.

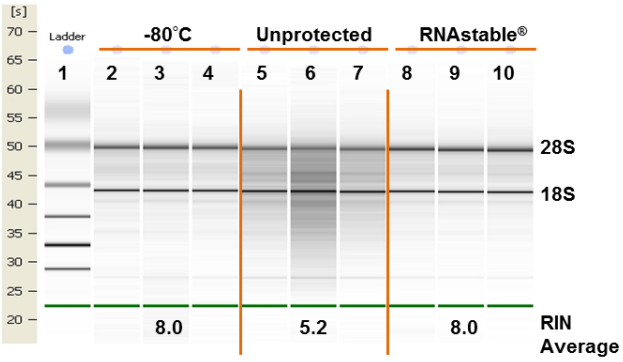

Figure 3: Bioanalyzer analysis of RNA integrity. Rehydrated RNA samples were run on an Agilent 2100 Bioanalyzer and the results recorded by the software. Lane 1 is the RNA ladder. Lanes 2 to 4 contain samples thawed following overnight storage at -80°C. Unprotected samples (lanes 5 to 7) or samples stabilized with RNAstable® (lanes 8 to 10) were analyzed following dehydration, overnight storage, and rehydration. Average RIN values are noted below each set of samples.

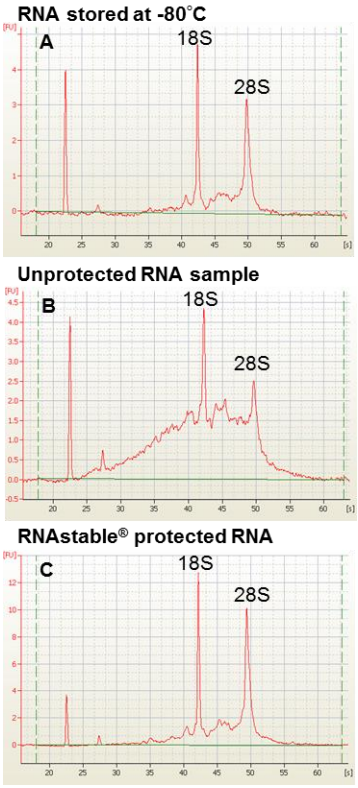

Figure 4: RNA quality of rehydrated RNA by Agilent 2100 Bioanalyzer electropherograms. Electropherograms for samples stored at -80°C (A), with no protection (B), or with RNAstable® LD (C) were reviewed to determine potential degradation of 18S and 28S RNA. Peak distribution and noise between the peaks were used to evaluate degradation. Example graphs are shown for each set of data.

SUMMARY

RNAstable® LD protects purified RNA dried in large volumes (i.e. 200 µL) from degradation during storage at ambient temperatures. These results indicate that RNA samples dried in RNAstable® LD maintain integrity after recovery, based on size analysis with gel electrophoresis (Figures 1 and 2) and confirmed by RNA Integrity Number (RIN) generated from the Agilent 2100 Bioanalyzer (Figures 3 and 4). The average RIN score of 8.0 of the RNAstable® LD stabilized RNA samples (Figure 3) is similar to that of the samples stored at -80°C, indicating high quality RNA. In contrast, RNA samples dried and stored without RNAstable® LD show a decrease in RIN score of about 3 units. The observation of two distinct bands of 18S and 28S subunits from electropherograms confirms the high quality of RNA samples protected by RNAstable® LD (Figure 4C). Biomatrica's innovative RNAstable® LD stabilization technology, with the 96-well deep well plate compatibility shown here, allows for convenient, room temperature protection of large volume, precious and labile RNA samples, ensuring the same high quality RNA preparation for costly experiments as provided by Biomatrica's standard small volume protocol.

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