

Application Forum

Stabilizing RNA at room temperature in RNastable[®]

Sharron Ohgi¹, Laurent Coulon, Rolf Muller, Judy-Muller-Cohn, and Omoshile Clement
Biomatrix, Inc., 5627 Oberlin Dr, #120, San Diego, CA 92121, USA.

Introduction

RNastable[®] is a novel preservation product developed to protect RNA from degradation during storage or shipment at ambient temperatures. The synthetic storage medium is based on the natural principles of anhydrobiosis (meaning “life without water”), a biological mechanism employed by some organisms that enables their survival while dry for more than 100 years. Anhydrobiotic organisms protect their DNA, RNA, proteins, membranes and cellular systems for survival in a dry state and can be revived by simple rehydration. RNastable was designed to mimic these unique characteristics to stabilize RNA at ambient temperatures for prolonged time periods. Quantitative RT-PCR analysis demonstrates successful amplification of RNA templates that were stored dry in RNastable for 29 months at room temperature and under accelerated aging conditions equivalent to 12 years of room temperature storage (elevated temperatures at 45°C). Samples were sealed inside a moisture-barrier bag including a desiccant pack to ensure ideal storage conditions. Rehydrated samples were used directly in reactions without further purification and exhibited no inhibition or loss of activity. This innovative technology prevents degradation of RNA at room temperature and offers tremendous cost and energy savings as an easy-to-use alternative to conventional freezer storage.

Experiment

Total RNA was extracted from frozen 293T cells using TRIzol[®] following the manufacturer’s instructions. Aliquots (500 ng) of extracted total RNA were added to RNastable[®] in a 96-well plate and allowed to dry overnight in a laminar flow hood. Dried samples were stored for 29 months both at room temperature inside a sealed moisture-barrier bag including a desiccant pack, and at 45°C.² Reference control samples were also stored at -80°C for the identical time period (29 months). RNA samples stored in RNastable were rehydrated in 25 µL DEPC-treated water to a final concentration of 20 ng/µL. Serial dilutions were performed to a final concentration of 2pg/µl. The unprotected samples were prepared in the same way. A 5-µL aliquot (10 pg) of each sample was used as template for expression of the 18S rRNA gene using the TaqMan[®] One-Step RT-PCR (ABI) kit. A final concentration of 400 nM was used for each forward and reverse primer in the reaction. A 250-nM final concentration of the 18S rRNA probe

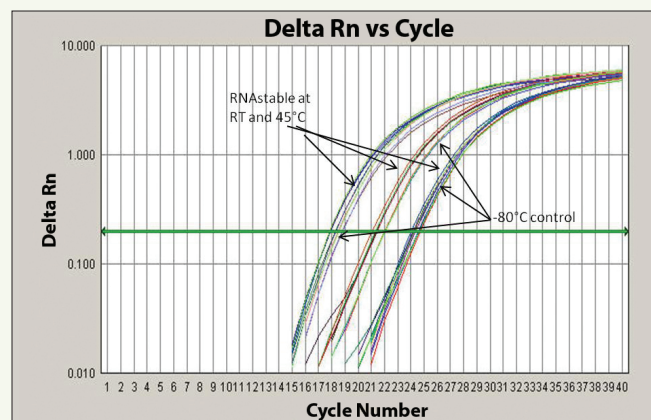


Figure 1. One-Step RT-PCR results of stored RNA samples in RNastable at room temperature and 45°C, versus storage at -80°C (control) quantified using the 18S rRNA gene. Three samples were amplified for each storage condition and the results from the 9 reactions are overlaid on the graph.

was used (5'-labeled with FAM and 3'-labeled with TAMRA). Reactions were prepared in a 25-µL final volume.

Results and discussion

The gel images,³ bioanalyzer RIN scores,³ and RT-PCR results (Figure 1) obtained for RNA samples stored in RNastable either at room temperature or at 45°C are similar to those at -80°C, indicating that RNastable offers same level of protection as that of cold storage. We find that optimal RNA sample protection from degradation at room temperature is afforded by storing in RNastable tubes or well plates and placed inside a heat-sealed moisture barrier bag with a desiccant. RNA recovery from RNastable is by simple hydration, and the sample is ready for use without further purification in downstream applications such as qRT-PCR, in vitro transcription, Bioanalyzer, and expression analysis. RNastable offers researchers the ability to store and transport RNA at room temperature without loss of sample quality, and at significant cost savings on materials, labor and shipping.

¹Author to whom all correspondence should be addressed (sohgi@biomatrix.com).

²Accelerated aging: 29 month storage at 45°C ≈ 12 years storage at room temperature.

³See: <http://www.biomatrix.com/rnastable.php>