

## RNA Stabilization at Room Temperature in RNAsable®

### Introduction:

RNAsable 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. RNAsable 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 RNAsable 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.

### Materials and Methods:

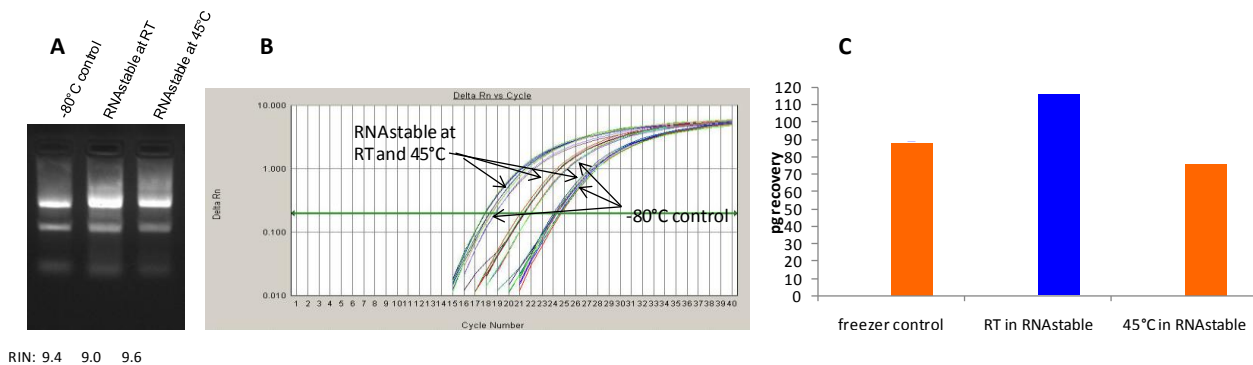
**Total RNA Isolation:** Human 293T cells were grown to 90% confluence in T-175 flasks in DMEM supplemented with 10% fetal calf serum at 37°C, 5% CO<sub>2</sub>. Cells were dissociated from the flask by incubation in 0.25% Trypsin-EDTA at 37°C for 5 min. The cell pellet was stored frozen at -20°C until needed. Total RNA was extracted from frozen 293T cells using TRIzol® following the manufacturer’s instructions. Isolated total RNA was resuspended in DEPC-treated water and stored at -80°C.

### Sample Preparation and Storage:

Aliquots (500 ng) of total RNA were applied to RNAsable in the 96-well plate format (catalog #90221-001) and allowed to dry overnight in a laminar flow hood. Dried samples were then stored for 29 months at room temperature inside a sealed moisture-barrier bag including a desiccant pack and at accelerated temperature of 45°C. Reference control samples were stored at -80°C for the identical time period.

### TaqMan® One-Step RT-PCR:

RNA samples stored in RNAsable 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 2 pg/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 was used (5’ labeled with FAM and 3’ labeled with TAMRA). Reactions were prepared in a 25 µl final volume.



**Figure 1:** (A) Aliquots of total RNA were stored for 29 months at either -80°C or at room temperature protected in RNAsable or unprotected. RNA integrity was assessed using agarose gel electrophoresis and bioanalyzer analysis (e.g. RIN score). (B) Aliquots of the stored samples were quantified using the 18S rRNA gene in a One-Step RT-PCR reaction. Three samples were amplified for each storage condition and the results from the 9 reactions are overlaid on the graph. (C) Recovery levels (pg) for total RNA stored under identical conditions.

**Results and Discussion:**

RNAstable allows for long-term stabilization of total RNA samples at room temperature with easy sample recovery by simple rehydration. Recovered RNA can be used directly without the need for further purification in downstream applications such as quantitative RT-PCR, *in vitro* transcription, bioanalyzer and microarray analysis. Optimal sample protection is afforded by storing samples in the presence of a desiccant and heat-sealing into a moisture-barrier bag (included with kit). RNA degradation due to fluctuating and inconsistent temperatures during shipment will be prevented, as samples are protected even after exposure to elevated temperatures for extended time periods when maintained in a desiccating environment. Samples can be prepared with minimal effort and shipped without expedited delivery fees or bulky dry ice containers. Such applications will be useful for sample transport to core facilities.