

Stabilization of poly(A) mRNA transcripts in RNASTable®

Introduction:

RNASTable is a novel stabilization product developed to protect RNA samples from degradation at room temperature. The technology is based on nature's principles of anhydrobiosis (meaning "life without water"), a biological mechanism used by some multicellular organisms that ensures their survival while dry for over 100 years. Anhydrobiotic organisms such as tardigrades and brine shrimp protect their DNA, RNA, proteins, membranes and cellular systems for survival and can be revived by simple re-hydration. By adapting these unique features to a novel synthetic storage medium, RNASTable was designed to stabilize RNA *dry* at ambient temperatures. This innovative technology offers protection of full-length poly(A) mRNA transcripts present in stored samples of purified total RNA. Studies demonstrate that mRNA is stabilized for up to **21 months at room temperature** and even at elevated temperatures (+50°C) for extended time periods. Samples recovered by a one-step rehydration protocol can be used directly in downstream applications with no inhibition or sample loss. The data presented below demonstrates that RNASTable protects labile full-length poly(A) transcripts from degradation and maintains its integrity during dry storage at ambient and elevated temperatures.

Materials and Methods:

Sample Preparation and Storage in RNASTable:

Total RNA was isolated from human 293 cells using the TRIzol® isolation protocol following manufacturer's instructions. The purified total RNA in DEPC-treated water was stored at -80°C in aliquots of 500 ng until ready for use. Aliquots (500 ng) of purified total RNA were applied directly into RNASTable in the 96-well format (Biomatrix catalog #90221-001) and allowed to dry overnight in a laminar flow hood. The unprotected samples were applied into empty wells and dried identical conditions. Samples were then stored for 21 months at room temperature in a heat-sealed moisture-barrier foil bag including desiccant packets and also at +50°C; frozen control samples were maintained at -80°C for the identical time period. Following storage, samples were recovered by rehydrating with 25 µl of DEPC-treated water, followed by immediate use in reactions for first-strand synthesis.

First-strand Synthesis and PCR amplification from RNA stored in RNASTable:

Total RNA (100 ng) was incubated with oligo dT at 65°C for 5 minutes. Samples were cooled on ice for 10 min to allow annealing. Reverse transcription was performed following manufacturer's instructions for the Superscript III® First Strand Synthesis Supermix kit (Invitrogen). A 2.3 kb amplicon was generated from PCR amplification. Aliquots (10 µl) of PCR reactions were run on a 0.8% agarose gel containing ethidium bromide.

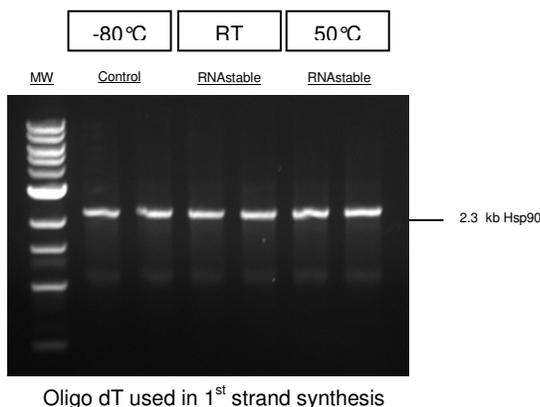


Figure 1: RNASTable protects poly(A) mRNA. Total RNA purified from 293 cells and stored dry in RNASTable at room temperature, 50°C and -80°C for 21 months was used in RT-PCR using Hsp 90 gene primers (2.3 kb). Expected amplicons were obtained from RNA stored in RNASTable, while unprotected and freezer-stored RNA resulted in reduced amplification of target products. Please note that the unprotected control stored at RT degraded at 3 months.

Results and Discussion:

Results show successful preservation of full-length poly(A) mRNA transcripts following 21 month dry storage in RNASTable. Amplification using oligo d(T) primers was significantly improved for samples protected in RNASTable as compared to unprotected samples and also frozen control samples (-80°C). Sample stability is secured even at elevated temperatures of +50°C for extended periods. These results indicate that full-length poly(A) mRNA transcripts, including medium copy number species such as Hsp 90, are protected from degradation when the purified total RNA



sample is stored dry at room temperature protected in RNAstable. Rehydrated RNA samples can be used directly in downstream applications and reactions without further purification, thus minimizing sample loss associated with typical purification protocols. RNAstable is compatible with downstream applications such as quantitative RT-PCR, *in vitro* transcription, microarray and bioanalyzer analysis. Biomatrica's innovative stabilization technology allows for convenient room temperature protection of precious and labile RNA samples. Protection against degradation is secured even at elevated temperatures, making sample transport convenient and reliable.

*For best results, it is recommended that samples stored over 1 month be kept either in a desiccating chamber or a heat-sealed, moisture barrier bag with a desiccant packet and stored at room temperature (15-25°C) with relative humidity $\leq 40\%$.