

RNA Stabilization at Room Temperature in RNASTable™

Introduction:

RNASTable is a novel RNA stabilization product developed to protect RNA samples from degradation dry at room temperature. RNASTable is based on the natural principles of anhydrobiosis (meaning “life without water”), a biological mechanism employed by some multicellular organisms that enables their survival while dry for up to 120 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 exploiting these unique characteristics, RNASTable was designed to stabilize RNA *dry* at ambient temperatures. This innovative technology offers tremendous cost and energy savings as an easy-to-use alternative to conventional storage at -20°C or -80°C and eliminates expensive shipments on dry ice. Studies demonstrate that RNA is stabilized for up to 6 months at room temperature and even at elevated temperatures (50°C), making RNASTable an ideal transport media for precious, labile RNA samples. The data presented demonstrates that RNA stabilized in RNASTable maintains its integrity during long-term dry storage at room temperature and even at elevated temperatures. Samples recovered by simple re-hydration can be used directly in downstream applications with no inhibition or loss due to degradation.

Materials and Methods:

Total RNA Isolation: Human 293T cells were grown to 90% confluence in T-175 flasks in DMEM supplemented with 1% fetal calf serum at 37°C, 5% CO₂. Cells were dissociated from the flask by incubating with 0.25% Trypsin-EDTA at 37°C for 5 minutes. The cell pellet was stored frozen at -20°C until ready for use. Frozen 293T cells were resuspended in 1 ml of PBS and total RNA was isolated using the TRIzol® isolation protocol following manufacturer’s instructions. Isolated total RNA was resuspended in DEPC-treated water and stored at -20°C.

Sample Preparation and Storage in RNASTable:

Aliquots of 50 µg and 100 µg of total RNA were applied to RNASTable in the 1.5 ml standard microfuge tube format (Biomatrixa catalog #93221-001) and allowed to dry for 1.5 hours in a SpeedVac® without heat. An unprotected control sample (NP) was prepared by drying 100 µg of total RNA into an empty tube under identical conditions. Samples were then stored for 6 months at room temperature with relative humidity of <50%.* RNA was re-hydrated by adding DEPC-treated water to a final concentration of 1 µg/µl for each sample. A 1 µg aliquot of each RNA sample (protected and unprotected control) was run on a 1.2% agarose gel containing ethidium bromide.

First-strand Synthesis from RNA stored in RNASTable:

293T total RNA stabilized in RNASTable were stored at room temperature or 50°C for 6 months with relative humidity of <50% prior to use as templates for first-strand synthesis. Each sample of total RNA (1 µg) was incubated with 300 ng of oligo dT at 65°C for 5 min. Samples were then cooled on ice for 10 min to allow annealing. Reverse transcription was performed using 50U of Stratascript™ Reverse Transcriptase and 40U of RNase Block. Samples were incubated at 42°C for 50 min to allow cDNA synthesis, and then incubated at 70°C for 15 min to inactivate the RNase inhibitor. A 1 µl aliquot of first-strand synthesis product was then used as templates for amplification of the human RNaseP transcripts. Aliquots of each reaction were run on a 1.2% agarose gel containing ethidium bromide.

Figure 1: Aliquots of 1 µg 293T total RNA stabilized in RNASTable and stored dry at room temperature with relative humidity <50% for 6 months. Samples were re-hydrated in DEPC-treated water and run on a 1.2% agarose gel. Lanes 1-8: 293T total RNA stored in RNASTable; NP: no protection control; positive control sample stored at -20°C.

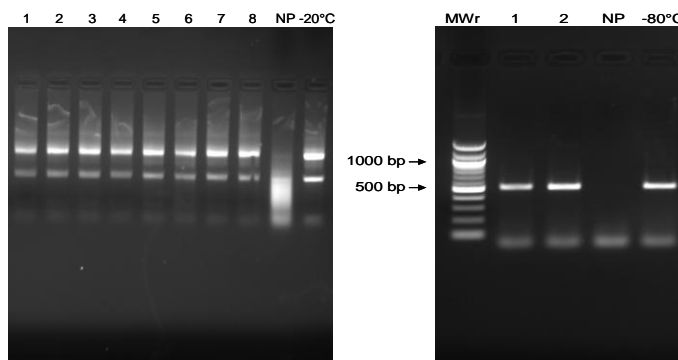


Figure 2: Aliquots of 500 ng 293T total RNA from 293T cells were stored in RNASTable for 6 months at room temperature or 50°C with relative humidity <50% and used as templates for first-strand synthesis and amplification of the RNaseP amplicon (517 bp). Lane 1: sample stored at room temperature; Lane 2: accelerated aging sample at 50°C; Lanes positive controls stored at -80°C; (-) negative control.

Results and Discussion:

RNASTable allows for long-term dry stabilization of precious, labile RNA samples at room temperature with easy sample recovery by simple re-hydration. Recovered RNA can be used directly without the need for further purification in downstream applications such as quantitative RT-PCR, *in vitro* transcription, microarray, bioanalyzer and hybridization analysis. Samples can be stored with minimal effort in shelves, drawers or boxes, greatly reducing reliance on costly freezer units. Fluctuating and inconsistent temperatures during shipment will not damage RNA stabilized dry in RNASTable. Sample stability is secured even at elevated temperatures of >50°C for extended periods. Biomatrixa’s innovative RNASTable ambient temperature storage technology thus eliminates the need for freezers and costly shipments in bulky dry ice containers, resulting in significant savings in cost, energy, space and time.

*For best results, it is recommended that samples stored over 1 month be kept either in a desiccating chamber or heat-sealed, moisture barrier bag with a desiccant packet and then stored at room temperature (15-25°C) with ≤50% relative humidity.