

microRNA (miRNA) Stabilization in RNAsable™

Introduction:

miRNAs play an important role in gene regulation. Genes encoding miRNAs are transcribed and then processed into mature single-stranded stem-loop structures that then bind to complementary mRNA transcripts to down-regulate gene expression. Dysregulation of miRNA expression and function has been associated with disease such as cancer and neurological disorders, and recently interest has broadened to cardiac research, virology, cell biology in general and plant biology. miRNAs comprise a sub-population of small RNAs contained within the total RNA content of a cell. Their low abundance demands highly-sensitive procedures and assays for their purification and detection. We have recently demonstrated protection of small miRNA species following long-term *dry* storage of purified total RNA in RNAsable, a novel room temperature storage medium designed to stabilize and protect labile ribonucleic acids from degradation. RNAsable protects by forming a thermo-stable barrier around RNA during the drying process. Dried samples can be stored in RNAsable for extended time periods and are even protected from degradation after prolonged exposure to elevated temperatures. Sample recovery requires simple rehydration and RNA is ready to use directly in downstream applications without further purification. Using a rehydrated sample derived from 293 cells, we successfully detected miRNA (hsa-miR-24) in a purified total RNA sample that was stored **dry for 18 months at 50 °C**. The data presented demonstrates that RNAsable successfully maintains the integrity of miRNA during dry storage of purified total RNA samples for long time periods.

Materials and Methods:

Sample Preparation and Storage:

Total RNA was isolated from human 293 cells using the TRIzol® isolation protocol (Life Technologies; Carlsbad, CA) following manufacturer's instructions. The isolated total RNA was stored in DEPC-treated water and stored at -80 °C in aliquots of 500ng. Aliquots of 500 ng total RNA were applied to RNAsable in the 96-well format (Biomatrixa catalog #90221-001) and allowed to dry overnight in a laminar flow hood. Samples were then stored for 18 months at 50 °C. Control samples were stored at -80 °C for the identical time period. Dried samples were rehydrated in 10 µl of DEPC-treated water to give a final concentration of 50 ng/µl and used immediately in downstream reactions.

First-strand Synthesis and qPCR amplification for detection of miRNA:

All samples, including controls, were serially diluted 10-fold to 5, 0.5 and 0.05 ng/µl and 5 µl of each dilution was used as template for reverse transcription using primers specific for hsa-miR-24 microRNA species with the *mirVana*™ miRNA Detection Kit (ABI; Foster City, CA). Detection of the highly expressed miRNA species is indicated by the successful amplification of a 90 bp amplicon. Quantitative PCR analysis of hsa-miR-24 microRNA was also performed on control and dry stored samples. Triplicate reverse-transcription reactions were performed using the TaqMan® MicroRNA Assay (ABI) that requires reverse transcription with a miRNA-specific primer, followed by real-time PCR with TaqMan probes.

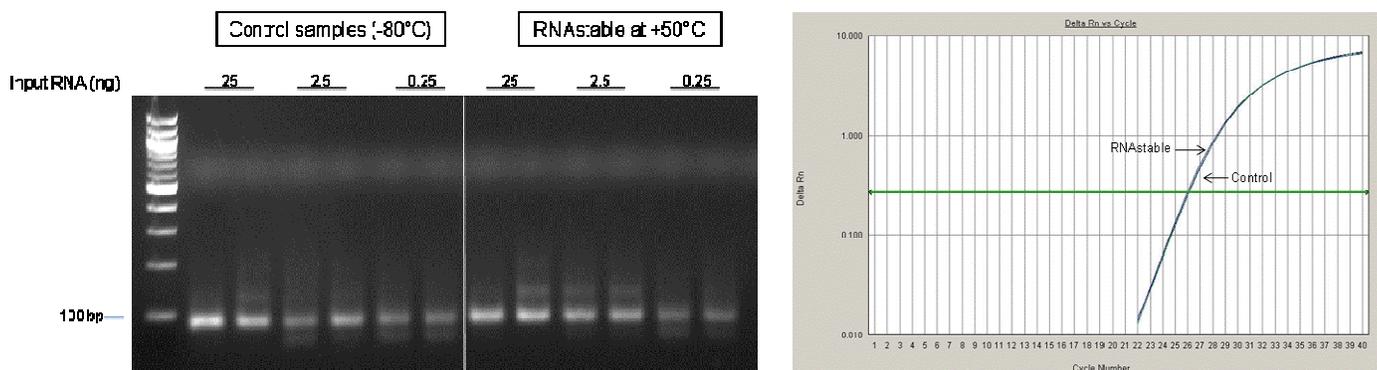


Figure 1. RNAsable protects miRNA. (*left*) Total RNA was purified from 293 cells and stored dry (500 ng) for **18 months at 50 °C**; control samples were stored at -80 °C for the identical time period. Detection of the highly expressed miRNA species is indicated by the successful amplification of a 90 bp amplicon. Results indicate that RNAsable can protect microRNA species within total RNA samples even after long-term exposure to elevated temperatures. These data suggest that miRNA can be successfully recovered from total RNA samples stored dry at room temperature in RNAsable for the equivalent of over 5 years using accelerated aging models. (*right*) Quantitative PCR analysis of hsa-miR-24 microRNA from total RNA samples (100 ng) purified from 293 cells and stored dry in RNAsable. Results indicate comparable recovery of hsa-miR-24 from total RNA samples stored either frozen (green) or dried in RNAsable and exposed to extremely elevated temperatures for long time periods (blue).

Results and Discussion:

RNAstable protects purified RNA samples, including small RNAs such as miRNA, from degradation during dry storage at ambient temperatures. Results indicate that hsa-miR-24 miRNA transcripts can be successfully recovered from total RNA samples stored dry in RNAstable for 18 months at 50°C, an equivalent of **over 5 years storage at room temperature** based on accelerated aging models. Rehydrated total RNA samples were used without further purification to remove RNAstable components and did not exhibit any interference or inhibition in downstream assays for detection of low abundance miRNA transcripts. Eliminating the need to further purify samples is particularly advantageous for detecting low quantity transcripts such as small RNAs. RNAstable offers a convenient ambient temperature sample storage and shipment strategy of precious and labile RNA samples that minimizes degradation and sample loss. Results presented above indicate that even low abundant rare transcripts, such as miRNAs, are protected in purified total RNA samples stored dry for extended time periods.