Reprint from

GEN Genetic Engineering & biotechnology News

Biotechnology from bench to business

Volume 27, Number 18 **October 15, 2007**

OMICS | Drug Discovery | Translational Medicine | Bioprocessing | Biobusiness

Drug Discovery Tutorial

Making RNA More Durable at Room Temp

New Storage Product for Preservation and Stability Is Based on Principle of Anhydrobiosis

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Each year millions of biological samples are processed, distributed, and stored worldwide. Currently samples such as DNA, RNA, proteins, bacteria, viruses, tissues, and other biological molecules are stored cold to prevent or reduce the rate of degradation. Even for small labs maintaining these cold environments requires multiple expensive refrigeration and freezer units, all of which greedily consume energy and limited laboratory budgets.

Current methods of sample transport are also problematic—as shipping frozen samples on dry ice is expensive, with shipments costing hundreds of dollars due to bulky containers and expedited delivery costs. Unfortunately even under carefully monitored cold storage environments, repeated freeze-thaw cycles and fluctuating temperatures only serve to promote degradation and compromise results.

All too often we are reminded that the power requirements necessary for a constant cold chain can be difficult to maintain through rolling blackouts, natural or man-made disasters, and the simple fact that only a small portion of the world can consistently supply power 24/7.

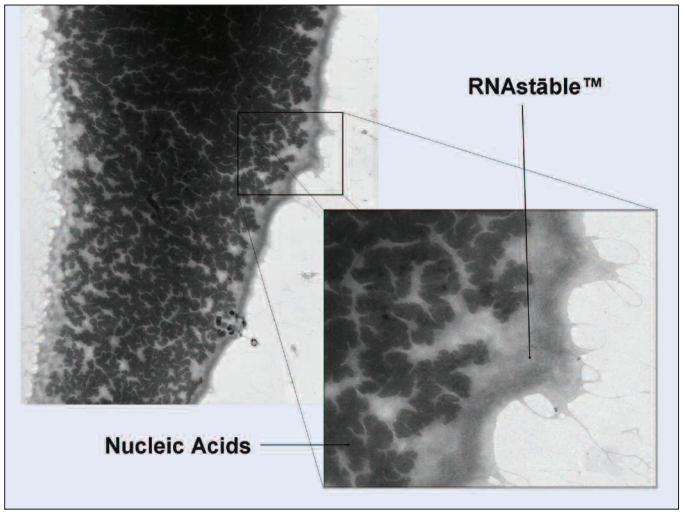

Figure 1. Electron micrograph of the RNAstable protective barrier. Electron microscopy shows the thermo-stable, glass-like shell that forms around nucleic acid molecules, which stabilizes and helps prevent degradation.

Power outages can lead to extensive and even insurmountable sample loss for individual labs, or even entire institutions, bringing into sharp focus the precarious nature of archived biological specimens. If a back-up freezer system is not available, precious samples are impossible to replace. The costs in economic terms are tangible, if not

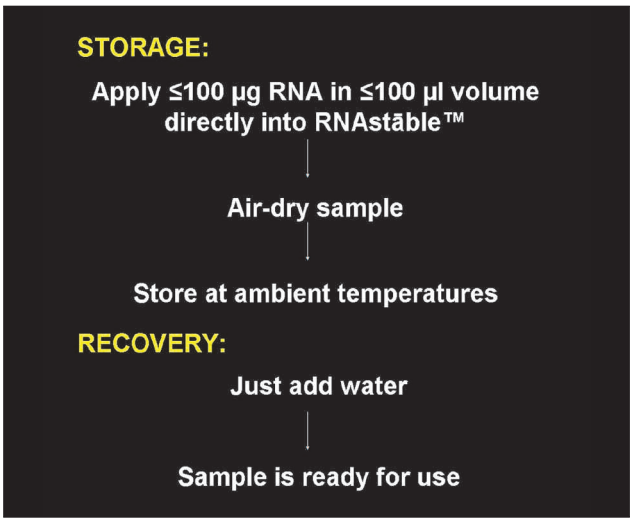

Figure 2. Protocol for RNA storage in RNAstable. RNA samples are applied directly into RNAstable, dried, and then stored at ambient temperatures. Sample recovery requires one-step rehydration, and the RNA is ready for use in downstream applications without the need for further purification.

downright painful, to researchers who could use these resources more productively elsewhere.

Despite all the precautions taken to keep samples cold, preservation is still not perfect. The average DNA sample, one of Nature's hardiest molecules, lasts for about a decade—not long enough if the sample is needed for future reference, as is the case for forensic samples.

Far more problematic are RNA samples, which are difficult to work with given their highly labile nature and tendency to degrade even under carefully controlled RNase-free conditions and cold storage. Even a short period of slightly elevated temperatures can compromise RNA integrity and detrimentally affect performance in downstream assays.

Interest in RNA is on the upswing due to its utility as a gene silencer and potential target for therapeutic drugs. A tremendous amount of research has also been devoted to its role in gene expression studies. Despite all of this, RNA remains decidedly scientist unfriendly. Once RNA is thawed, a certain anxiety overcomes the scientist to make sure everything is done quickly before it degrades. Current methodologies are limited to storing RNA, either purified or in tissue, in cold environments; until recently there were no products that stabilized RNA at room temperature.

From Nature to the Lab

Biomatrica (www.biomatrica.com) has developed a biostabilization technology to prevent the degradation of biological materials at room temperature, which eliminates the need for cold storage and shipping.

RNAstable™ directly preserves and stabilizes RNA samples at ambient and elevated temperatures. RNAstable is based on the natural principles of anhydrobiosis (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 can protect their DNA, RNA, proteins, membranes, and cellular systems for survival and can be revived by simple rehydration.

Biomatrica's technology transfers the molecular principles of anhydrobiosis to a synthetic chemistry-based stabilization science that was used to develop RNAstable to prevent RNA degradation and stabilize the fragile molecule at ambient temperatures. RNAstable works by forming a glass-like shell, essentially shrink-wrapping RNA samples and providing protection against degradation (*Figure 1*).

Method of Use

RNAstable is supplied as a dried matrix on the bottom of tubes or in a 96-well format. Each sample-storage tube or well contains enough matrix to protect up to 100 µg of RNA. The steps involved in using RNAstable for RNA storage are outlined in *Figure 2*.

By adding RNA in either water or buffer, RNAstable is rehydrated and mixes with the RNA. Through its natural affinity to RNA, RNAstable associates with the nucleic acid in the liquid phase. Air-drying of the mixture results in a stabilizing glass that serves to protect the RNA from degradation. Once completely dried, samples can be stored at room temperature and relative humidity conditions ≤50% or stored in a moisture barrier container.

Sample recovery requires rehydration using water or a buffered solution. Since the rehydration volume can be chosen between 10 and 100 µL, storage of RNA in RNAstable also provides a method for sample concentration, eliminating the need for time-consuming salt precipitations and sample loss due to multiple wash steps or microconcentration columns.

RNA samples recovered following storage in RNAstable can be used directly in downstream applications such as reverse transcription, cDNA synthesis, PCR, gel electrophoresis, Northern blotting, hybridization analysis, and bioanalyzer and microarray analysis without inhibition or interference.

Stabilization of RNA

To validate the stabilization of RNA stored dry in RNAstable, total RNA was isolated from human 293T cells grown to 90% confluence in T-175 flasks in DMEM supplemented with 1% fetal calf serum at 37°C and 5% CO_2. 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.

Aliquots of 50 µg and 100 µg of total RNA were applied to RNAstable and allowed to dry for 1.5 hours in a SpeedVac® without heat. An unprotected control sample was prepared by drying 100 µg of total RNA into an empty tube under identical conditions. Samples were then stored for 14 days at room temperature or four months at 50°C to assess long-term stabilization. RNA was rehydrated by adding DEPC-treated water to a final concentration of 1 µg/µL for each sample.

Aliquots of RNA stored at room temperature in RNAstable and control samples stored at -80°C were analyzed with a bioanalyzer. Results indicate that RNA is stabilized and protected in RNAstable, as there is no apparent degradation when compared to the frozen control sample (*Figure 3*, *left panel*).

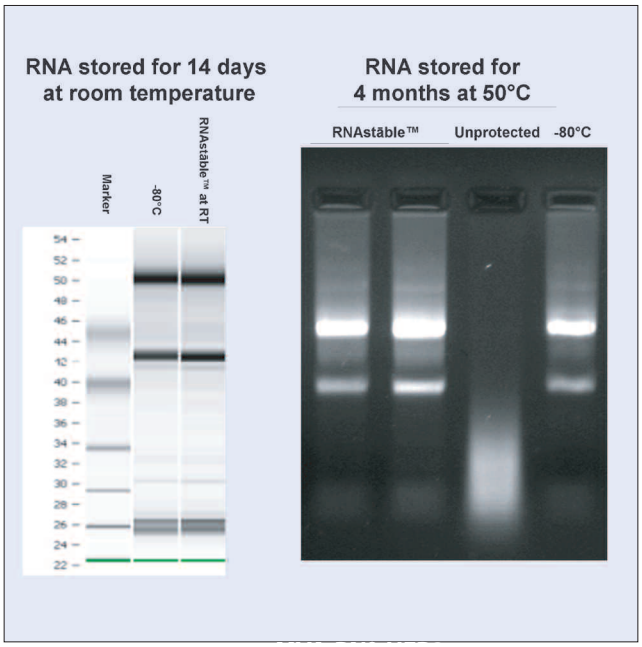

Figure 3. RNAstable protects RNA from degradation. RNA samples stored dry either at room temperature or at elevated temperatures for long time periods are stabilized and protected from degradation. Results from bioanalyzer analysis indicates no detectable degradation of samples stored in RNAstable after storage for two weeks at room temperature as compared to control samples (left). Samples stored in RNAstable were protected from degradation for four months at 50°C, while RNA stored unprotected was completely degraded (right).

RNAstable also functions to protect RNA at elevated temperatures, even after four months at 50°C. Sample storage at 50°C for four months is equivalent to over two years at room temperature. RNAstable samples did not appear degraded compared to freezer-stored control samples, while the unprotected sample was completely degraded (*Figure 3, right panel*).

RNAstable allows for long-term dry stabilization of RNA samples at ambient temperatures with sample recovery by rehydration. Recovered RNA can be used directly without the need for further purification in downstream applications. 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. GEN