

RNAstable Handbook

Preserve and store RNA samples at room temperature

Protocols for Sample Stabilization, Sample
Recovery and Downstream Applications

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Product Formats

RNAstable™

Format*	Found in Catalog Number	Description	Usage
Individual tubes	<ul style="list-style-type: none"> • 93221-001 • 93220-001 	<ul style="list-style-type: none"> • 1.5 ml screw cap microfuge tubes made of durable polypropylene 	Individual samples
96-well plate	<ul style="list-style-type: none"> • 90221-001 • 93220-001 	<ul style="list-style-type: none"> • 96-well plate made of durable polypropylene • Standard footprint • Hinged lid provides additional sample protection 	Low & High Through-put labs

*All formats are supplied with barcodes. For information on labels with RFID (Radio Frequency Identification Device), please contact us.

* All formats identical capacity, ≤100µg RNA

Accessories

- SamplePouches™ Moisture-barrier foil bags sold in packs of 12 (Catalog number 14001-007).

Introduction

Biomātrica offers innovative technologies for stabilizing biological samples at room temperature. We have developed a novel platform technology designed for use in protecting complex biological samples and assays. Sample preservation is achieved without degradation, thus enabling labs to decrease their reliance on freezers and drastically reduce shipping costs.

RNAstable is a unique storage medium that preserves RNA samples at room temperature. RNAstable allows for long-term stabilization of RNA samples with easy sample recovery by simply adding water.

RNAstable is based on the natural principles of anhydrobiosis (“life without water”), a biological mechanism employed by organisms such as tardigrades and brine shrimp that enables their survival while dry

for up to 120 years. Anhydrobiotic organisms protect their DNA, RNA, proteins, membranes and cellular systems, and can be revived by rehydration. By exploiting these unique characteristics, RNAstable preserves RNA dry at ambient temperatures. RNAstable works by forming a glass-like shell, securely “shrink-wrapping” RNA samples and protecting against degradation.

RNAstable enables you to store RNA samples for months and allows you to use samples directly in downstream applications without the need for further purification. There is no sample degradation, resulting in increased reproducibility and reliability. Since samples are stored at room temperature, precious freezer space is freed up. The cost for shipping RNA samples is drastically reduced, as samples secured with RNAstable can be transported at ambient temperatures, even for extended transit times.

Using RNAstable, you can completely recover your RNA samples by simple rehydration - *just add water*. In addition, you can concentrate your sample during recovery. Rehydrated samples are ready for immediate use, without the need for further purification. Downstream applications using recovered samples include:

- qRT-PCR
- Bioanalyzer and microarray analysis
- End-point PCR and gel analysis
- cDNA synthesis
- Reverse transcription

Visit www.biomatrix.com for further details on the complete line of room temperature sample stabilization products that Biomatrix offers.

Principles and Procedures

RNAstable is a mixture of dissolvable compounds that stabilizes RNA at room temperature. The matrix formulation is based upon the natural principles of anhydrobiosis and synthetic chemistry. Meaning

“life without water,” anhydrobiosis is a biological mechanism employed by some multi-cellular organisms that enables their survival in a dry state for periods up to 120 years (Crowe et al., *Ann. Rev. Physiol.* 1998. 60:73); during these extended dry periods, proteins, DNA, RNA, membranes, and cellular systems are protected and can be revived by rehydration.

Based on the unique thermo-stable properties of Biomatrix’s proprietary formulation, RNASTable forms a protective seal around RNA as it dries, effectively “shrink-wrapping” the sample in a protective coating. Drying can occur at ambient temperatures, utilizing a SpeedVac®. Stored dry at ambient temperatures, the protected RNA can be safely stored for extended time periods. Currently, Biomatrix has >6 months of real-time data on stable storage of RNA in RNASTable; accelerated aging studies indicate that RNA can be safely stored for up to 2 years at room temperature. RNASTable can even protect RNA kept at 50°C for up to 4 months. Samples can be recovered through simple rehydration and are ready for immediate use, without the need for further purification.

Storage

RNASTable products and kits should be stored dry in their original unopened packaging at ambient laboratory temperatures (*i.e.* room temperature at 15-25°C or 59-77°F) and at a relative humidity of ≤50%.

The RNASTable Trial Kit (catalog number 93220-001) and RNASTable 96-well Plate Kit (catalog number 90221-001) are supplied with moisture-barrier foil bags (SamplePouch Catalog number 14001-007). The SamplePouch allows for storage even in uncontrolled environments with elevated relative humidity above 50%. The moisture barrier foil bag should be opened just before use and, prior to storage, completely closed using the re-sealable closure. Store any unused products in their original packaging at room temperature in a clean, dry environment.

When using the RNAstable Tube Kit (catalog number 93221-001), please store the stabilized samples, or the entire Tube Kit container, in a desiccating chamber to ensure that the relative humidity is $\leq 50\%$.

For optimal results when shipping samples stabilized in RNAstable tubes or 96-well plates, please utilize the moisture barrier foil bag (SamplePouch). These can be purchased separately.

Note: Relative humidity above 50% for extended periods of time will significantly reduce product performance and sample protection in RNAstable. Climate-controlled laboratory environments and buildings are normally maintained at 40-50% relative humidity levels. To ensure relative humidity levels of $\leq 50\%$ and optimal results, please store samples in either the moisture barrier foil bag (provided with the 95-well plate and Trial Kit), or a desiccating chamber.

Please contact Biomatrix for information on desiccating chambers customized for the RNAstable product.

Applications

Sample Type:

RNAstable has been used extensively for room temperature dry storage of total RNA and poly(A) mRNA.

Assay Type

RNA stored in RNAstable is ready for immediate use in the following applications:

- Quantitative RT-PCR
- Bioanalyzer analysis
- Agarose gel electrophoresis
- Reverse Transcription (RT)
- RT-PCR
- Microarray analysis
- cDNA synthesis

Shipping:

RNAstable provides the ideal format for transport and shipping of samples at ambient temperatures. Individual tubes or plates can be slipped into moisture barrier foil bags (SamplePouches) and shipped

conveniently without the need for cold packs, dry ice or Styrofoam[®] packing, thus greatly reducing shipping costs. Fluctuating temperatures or delays during transport do not affect samples protected in RNAstable. (Additional SamplePouches are available for purchase. Visit www.biomatrix.com for complete product listings and details.)

Important Notes

Please take a few moments to read this handbook carefully before beginning the sample stabilization step for dry storage of RNA at room temperature. For optimal protection and sample recovery, RNA should be purified of any contaminating RNase activity.

Sample Application and Drying

Prior to applying to wells or tubes containing RNAstable, samples should be resuspended in DEPC-treated water. Sample must then be dried completely for optimal protection and stability during room temperature storage. Sample volumes of up to 100 μ l of water can be applied directly into an RNAstable tube or well and dried under vacuum (e.g. using a SpeedVac). For optimal results, do not exceed 100 μ g of total RNA per tube or well in a maximum volume of 100 μ l water.

Sample Recovery

To recover samples stored in RNAstable, just add water. An aliquot of DEPC-treated nuclease-free water is supplied with the kit. Samples are then ready for immediate use in downstream applications. It is not necessary to further purify rehydrated samples. Aqueous solutions such as TE buffer (10 mM Tris, 1 mM EDTA), PCR reaction buffers, and reverse transcription buffers are also compatible with the recovery of samples from RNAstable.

Concentrate without loss

Storage of RNA samples dried into RNAstable is an easy and efficient means for concentrating RNA since the rehydration volume can be chosen between 10-100 μ l. Eliminate the need for time-

consuming salt precipitations and reduce sample loss associated with multiple wash steps or microcolumn concentrators by using RNAstable to store and then recover precious RNA samples.

Protocol: Sample preparation

This protocol is designed for the preparation of purified RNA for storage in RNAstable.

Types of RNA

Suitable types of RNA that can be stored in RNAstable include total RNA purified from cells or tissues, and poly(A) mRNA.

Purification Techniques

Most standard molecular biology techniques and/or commercially available kits are compatible with RNAstable storage. For optimal results, RNA samples should be RNase free.

Purified RNA that is RNase-free should be resuspended in DEPC-treated water prior to application into RNAstable.

Determining yield

The concentration of the RNA sample should be determined prior to sample application into RNAstable. Although not essential, applying a known amount of RNA into RNAstable™ for storage can facilitate sample retrieval and subsequent applications.

For optimal protection in RNAstable™, do not apply more than a total of 100 µg of RNA per well or tube in a maximum volume of 100 µl water. Samples must be *completely* dried before storage at room temperature to prevent degradation; we recommend drying samples under a vacuum (e.g. SpeedVac).

Protocol: Sample drying and storage

RNAstable preserves RNA samples at room temperature. Each tube or plate contains RNAstable provided as a coating at the bottom of the tube or well, which protects picogram to microgram amounts of RNA. RNAstable is formulated so that upon application of liquid samples, the medium dissolves and forms a protective coating around the RNA. The sample must then be completely dried for maximum protection and stability for storage at ambient temperatures.

Note: In the event you want to determine the RNA concentration at a later date using a spectrophotometer, you should keep an unused RNAstable well or tube to generate a blank. See pg. 16 for further instructions.

Procedure

- 1. Determine the amount of purified RNA ($\mu\text{g}/\text{ml}$) in the sample and calculate the amount to be applied into RNAstable wells or tubes.**

RNA should be resuspended in DEPC-treated water prior to application on RNAstable.

- 2. Open cap on the tube or remove the seal from the 96-well plate. Gently add up to 100 μl [$\leq 100 \mu\text{g}$] of the sample directly into the center of each tube or well containing RNAstable.**

For optimal sample protection and recovery, the final volume of the sample applied to each well should be $\leq 20 \mu\text{l}$; do not exceed applying 100 μl water per tube or well.

- 3. Mix the sample thoroughly with gentle pipetting (avoid forming air bubbles).**
- 4. Leave the tube or 96-well plate open to dry overnight in laminar flow hood.**

For volumes $\geq 20 \mu\text{l}$, or for accelerated drying, please use a vacuum **with no heat** to ensure complete sample drying (e.g. SpeedVac). Recommended drying times are provided in Table 1 (pg. 10).

Note: Drying should occur at ambient laboratory temperatures (20°C – 25°C) with relative humidity below 50% (see pg. 5).

Table 1. Drying times with SpeedVac (no heat)

Sample Volume	Drying Times
10 μl -20 μl *	30 minutes
>20 μl -30 μl	1 hour
>30 μl -100 μl	1.5 hours

*RNAstable is designed for optimal protection and recovery for sample volumes of $\leq 20 \mu\text{l}$.

5. Cover samples after drying and store at room temperature in a sealed moisture barrier foil bag.

After complete sample drying, plates should be re-sealed with aluminum foil seals (provided in kit and available for separate purchase). Tubes should be tightly closed using screw cap.

RNAstable is sensitive to humidity, therefore it is critical to maintain stored samples in an environment with relative humidity below 50%. Store all samples in sealed moisture barrier foil bags.

Protocol: Sample recovery

RNA stored in RNAstable can be recovered by the addition of water or aqueous buffer. Samples are ready for downstream applications *without the need for further purification.*

Procedure

- 1. Add 10-100 μ l of DEPC-treated water (supplied in kit) or aqueous buffer directly to the dried sample in RNAstable.**

Individual wells in a plate can be opened by puncturing the aluminum foil seal with a pipette tip or razorblade.

Samples may be rehydrated directly with aqueous buffers used for downstream applications such as reverse transcription buffers, PCR buffers, etc.

Note: Samples can be easily concentrated at this step if an appropriate amount of water or buffer is used to re-hydrate a known amount of stored RNA.

- 2. Incubate at room temperature for 15 min to allow complete rehydration.**

After 15 minutes, mix the sample by gently pipetting up and down to resuspend the sample. Avoid forming bubbles.

- 3. The rehydrated sample is now ready for use directly in downstream applications.**

To determine RNA concentration and recovery yield, see **Appendix A** (pg. 16) for directions on how to perform UV spectrophotometry (*i.e.* A_{260} reading) using samples stored in RNAstable.

- 4. Store unused rehydrated samples at 4°C or room temperature for up to 3 days.**

Rehydrated samples in water containing RNAstable can be re-dried without loss of efficient sample stabilization. Repeating this process more than 3 times is not recommended.

Protocol: Downstream applications

Rehydrated RNAstable™ samples do not require further purification for the majority of molecular biology applications, and can be used directly without interference or inhibition in downstream applications. See pg. 6 for complete list of suitable applications. For further results and examples, refer to www.biomatrix.com under *Application Notes*.

RT-PCR and quantitative RT-PCR analysis using TaqMan or SYBR® Green reagents should be performed using guidelines as detailed in **Appendix B** (pg. 18).

If necessary, samples can be purified using column purification technology from commercially available kits.

Troubleshooting Guide

Dry storage of RNA in RNAstable is extremely effective for storage of samples at room temperature, if attention is paid to ensure complete drying and proper storage conditions (*i.e.* between 15-25°C with relative humidity $\leq 50\%$). All samples should be placed in the moisture-barrier bag for storage as significant moisture content in the air will hydrate RNAstable, resulting in sample degradation.

The following troubleshooting guide may be helpful in solving any problems that arise. Scientists at Biomatrixa's Technical Service Department are available to answer questions about the information and protocols in this handbook or general molecular biology applications (see pg. 21 for contact information).

Situation	Comment	Suggestion
RNA is currently in TE buffer.	RNA must be in water prior to application into RNAstable.	Precipitate RNA and resuspend in DEPC-treated water.
Added too much water or TE buffer for rehydration.	Addition of too much water or TE buffer does not harm RNA or RNAstable.	Place hydrated sample in laminar flow hood and allow to partially or fully dry. Use partially dried sample or re-hydrate fully dried sample in correct volume.
Unused RNAstable color is faded or has a slight change in coloration.	Fading or color change of RNAstable does not affect protective properties.	Proceed with sample application, drying, storage and rehydration as directed.
Low yield of rehydrated RNA sample.	Purified RNA safely stored in RNAstable does not degrade unless it is contaminated by considerable quantities of RNases. If recommended sample preparation, drying, and storage procedures are followed, degradation from contaminating RNases will be inhibited.	<ul style="list-style-type: none"> • Prior to sample application, record the quantity of RNA applied to RNAstable for future reference. • Make sure sample is applied to center of well or tube containing RNAstable and not on the sides of the vessel. • Identical samples can be rehydrated separately and the contents can be combined. • Ensure samples are RNase free prior to storage.

Situation	Comment	Suggestion
RNAstable™ turned pink after addition of RNA sample.	RNAstable contains a color indicator. The color change will not inhibit protection.	Proceed with protocol.
Cold storage of dried samples at (4° or -20° or -80°C).	Cold storage of samples in RNAstable does not interfere with product performance.	Samples can be placed at room temperature.
The desired volume for rehydration is outside of the recommended minimum or maximum volume.	<ul style="list-style-type: none"> • It is necessary to remain within the recommended 10-100 µl rehydration volume for optimal results. • Lower volumes can inhibit complete rehydration of RNA and decrease recovery. • Larger volumes may result in over-flow of wells in the 96-well format, and impede proper mixing. 	Samples can be rehydrated in the recommended volumes and then brought up to the desired volume once samples are mixed. Transfer to a separate vessel may be necessary.
RNA is contaminated.	Only purified RNA that is free of RNase should be used for long-term storage in RNAstable.	Rehydrated RNA can be purified using organic extraction (phenol: chloroform:IAA) or column purification technology from commercially available kits.
RNA appears degraded after rehydration.	Dry storage of RNA in RNAstable is dependent on low humidity environments (<i>i.e.</i> ≤50%).	All samples should be stored inside sealed moisture barrier foil bags and kept at room temperature.
Reduced or increased rehydration times.	Minimum rehydration time (15 min) is important for full recovery of stored RNA. Maximum rehydration time should not exceed 1 hour. A 5 min rehydration time with mixing can be used; however, there may be a slight reduction in sample recovery.	Rehydration of 15 min is recommended to ensure complete sample recovery. Keep plate or tube covered with lid during rehydration to avoid contamination and evaporation.

Situation	Comment	Suggestion
<p>Less volume is recovered from well than was initially added for rehydration.</p>	<p>Rehydration of the dissolvable matrix may cause a reduction in sample volume recovery. This is especially noticeable for smaller volumes (i.e. <20 μl)</p>	<ul style="list-style-type: none"> • Minimal loss of recovered sample volume does not affect the stability or performance of RNA or RNAstable in downstream applications. • A 10% loss in volume can be included for adjustment (e.g. add 11 μl of water to ensure recovery of 10 μl of rehydrated sample).

Appendix A: UV spectrophotometry with RNAstable

The absorbance of UV light (260 nm) can be used to determine the concentration of rehydrated RNAstable samples.

Procedure

1. Prepare sample and blank

The UV spectrophotometer must be blanked against an aliquot of RNAstable for accurate A_{260} readings.

Sample: Re-hydrate well or tube containing sample stored in RNAstable in a final volume large enough to obtain an accurate absorbance reading (volume is dependent on cuvette size). The absorbance will be accurate up to a reading of 2, at which point sample dilution may be necessary to obtain an accurate A_{260} reading.

Blank: Blank the spectrophotometer with an RNAstable-only reference sample. Re-hydrate an unused tube or well containing only RNAstable (*i.e.* no RNA) using the same volumes (including dilutions) as was performed for sample preparation.

2. Reference the spectrophotometer against RNAstable.

Blank the spectrophotometer using the RNAstable-only reference sample.

3. Determine A_{260} reading of sample.

4. Calculate concentration of sample.

The nucleic acid concentration for a standard cuvette with 1 cm path length can be calculated as follows:

$$\text{Conc. } (\mu\text{g/ml}) = (A_{260} \text{ reading}) (40 \mu\text{g/ml}) (\text{dilution factor})$$

Possible sources of error in determining RNA concentration can include contamination with proteins and organic molecules, such as phenol and ethidium bromide. If this is the case, the RNA can be purified prior to absorbance reading (e.g. put through a spin column). An alternative method, such as gel visualization, can also be used.

Appendix B: Quantitative PCR using samples stored in RNAstable

Samples recovered from dry storage in RNAstable can be used directly in real time quantitative PCR (QPCR) reactions without further purification. For optimal results we recommend the following dilutions of rehydrated samples when using either TaqMan® or SYBR® Green technology.

TaqMan® Technology

A 1:5 final dilution is recommended. Samples should be rehydrated in a volume appropriate for 50 µl QPCR reaction (total final volume) prior to following manufacturer's instructions.

Example TaqMan® One-Step RT-PCR Master Mix Reagents Kit

1. Human 293T total RNA was diluted to 100 ng/µl in DEPC-treated water and 5 µl was added to wells containing RNAstable that were then dried, sealed and stored at room temperature for 6 weeks inside a moisture barrier foil bag.
2. Samples were rehydrated with 50 µl of water for 15 min at room temperature and mixed gently prior to use.
3. For each QPCR reaction, 10 µl of rehydrated sample was added to 40 µl of QPCR master mix containing:

25 µl 2X Master Mix without UNG (ABI)
1.25 µl 40X Multiscribe and RNase Inhibitor Mix
1 µl 10 µM forward primer
1 µl 10 µM reverse primer
1.25 µl 10 µM probe
10.5 µl water
+10 µl rehydrated RNA sample
50 µl total volume

4. A Thermal Cycler (ABI 7300 Real Time PCR System) was used with the following cycling conditions:

Reverse transcription:	48°C for 30 min
AmpliTaq gold activation:	95°C for 10 min
PCR (40 cycles):	95°C for 15 sec 60°C for 1 min

5. The predicted concentration of RNA as determined by QPCR was found to be equivalent between the test and control samples (+/-10%). A slope of -3.1 to -3.6 and an R² value of 0.99 are considered acceptable.

SYBR[®] Green Technology

A final 1:20 dilution is recommended for RNAstable samples used in QPCR with SYBR[®] Green reagents.

Example SYBR[®] Green reaction

1. Human 293T total RNA was diluted to 100 ng/μl in DEPC-treated water and 5 μl was added to wells containing RNAstable that were then dried, sealed and stored at room temperature for 6 weeks inside a moisture barrier foil bag.
2. Samples were rehydrated with 25 μl of water for 15 min at room temperature prior to use.
3. For each QPCR reaction, 2.5 μl of rehydrated sample was added to 50 μl of QPCR master mix following manufacturer's instructions
4. Use thermal cycling reaction conditions as recommended by the manufacturer.

Quality Control

Every manufacturing production lot of RNAstable is quality control tested for contamination and functional performance. All products are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

RNAstable technology is specifically designed for the stabilization of purified RNA samples. No claim or representation is intended for their use on any other biological materials. RNAstable products are for *research use only* and are not intended for use in diagnostic procedures. For optimal performance, products must be used and stored according to manufacturer's guidelines.

Optimal protection of RNA in RNAstable occurs when samples are prepared and stored at room temperature and in a relative humidity environment $\leq 50\%$. Please refer to "Sample drying and storage" (pg. 9) for details on preparing and storing RNA under relative humidity conditions exceeding 50%.

Warranty and Satisfaction Guarantee

Biomatrix, Inc. guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for a particular use. Should any of our products fail to perform satisfactorily due to any reason other than misuse, Biomatrix will replace it free of charge or refund the purchase price, at your request. Biomatrix reserves the right to change, alter, or modify any product to enhance its performance and design. Contact Biomatrix if a product does not meet your expectations.

Warranties of merchantability or fitness for a particular purpose are expressly disclaimed. Biomatrix's liability shall not exceed the purchase price of the product. Biomatrix shall have no liability for indirect, consequential, or incidental loss or damages from the use, results of use, or inability to use its products. A copy of Biomatrix's full limited warranty statement is available at www.biomatrix.com or upon request.

Technical Assistance

Biomatrica, Inc. takes pride in providing efficient quality technical support. Biomatrica's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of Biomatrica's biostability and storage products. Please contact Biomatrica directly with any questions regarding RNAstable technology, product use, or general matters.

Technical Service Department:

Phone: USA (866) DRY-MTRX or (866) 379-6879

Web: www.biomatrica.com

Email: support@biomatrica.com

Literature Citation

When a procedure utilizing this product is described in a manuscript for publication, kindly refer to it as RNAstable from Biomatrica, Inc.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective eyewear. For more information, please consult appropriate material safety data sheets (MSDS, available online at www.biomatrica.com, in PDF format).