

RNAstable Handbook

Preserve and store RNA samples at room temperature

Protocols for Sample Stabilization, Sample
Recovery and Downstream Applications

Biomātrica[®]
THE BIOSTABILITY COMPANY

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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 per tube or well

Accessories for Long-term Sample Storage >1 month

Moisture Barrier Bags:

Sample Pouches with Desiccant Packs, Catalog number **14001-007**. Sold in sets of 12.

Dry Storage Cabinets:

- **Dry Storage Cabinet, Small with racks**, Catalog number: **95905-058**. External dimensions: 6"x 6"x10". Maximum capacity of (10) multi-well plates. Includes (1) desiccant pack.
- **Dry Storage Cabinet, Medium with racks**, Catalog number: **95904-158**. External dimensions: 7"x 24"x 10". Maximum capacity of (50) multi-well plates. Includes (5) internal adjustable racks, hygrometer, (1) desiccant pack.
- **Dry Storage Cabinet, Large with racks**, Catalog number: **95905-158**. External dimensions: 7"x 24"x 20". Maximum capacity of (100) multi-well plates. Includes (10) internal adjustable racks, hygrometer, (2) desiccant packs.
- **Dry Storage Cabinet, Medium without racks**, Catalog number: **95904-168**. External dimensions: 7"x24"x10". Maximum capacity of (50) multi-well plates. Box only.
- **Dry Storage Cabinet, Large without racks**, Catalog number: **95904-178**. External dimensions: 7"x24"x20". Maximum capacity of (100) multi-well plates. Box only. **Adjustable Cabinet Rack**, Catalog number: **95904-058**.
- Adjustable rack for dry storage cabinets. Fully adjustable, holds tubes, plates, various size boxes. Hold up to (10) multi-well plates. Dimensions: 4"x5"x8"

Introduction

Biomatrix offers innovative technologies for stabilizing biological samples at room temperature. We have developed a high performing 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. Samples are stabilized without cold storage and there is no sample degradation through freeze/thawing. This results 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 periods.

Using RNAstable, you can completely recover your RNA samples by simple rehydration - *just add water*. Rehydrated samples are ready for immediate use, without the need for further purification.

Downstream applications using recovered samples that have been tested include:

- Quantitative RT-PCR

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

Additional applications are certainly feasible as we have found no significant difference in those applications tested.

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

Principles and Procedures

RNAstable is a mixture of thermo-stable dissolvable compounds that stabilize RNA at room temperature and prevents degradation. 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 of more than 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 Biomatica’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 either through airdrying (for example in a biosafety hood) or with a vacuum concentrator (*i.e.* SpeedVac[®]). Stored dry at ambient temperatures, the protected RNA can be safely stored for more than 10 months. Biomatica accelerated aging study demonstrate safe stored for up to 5 years at room temperature and RNAstable can even protect RNA kept at 50°C for up to 10 months. Please note that we recommend storing prepared samples protected from moisture in either a dry storage cabinet or a heat-sealed, moisture-barrier bag along with a silica gel desiccant packet. 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) until ready for use.

The RNAstable products are supplied in moisture-barrier foil bags along with a desiccant pack. An extra bag and desiccant set are also included. Additional Sample Pouches/Desiccant Packs are sold as sets of twelve at www.biomatrica.com, catalog number 14001-007. The sample pouch allows for dry storage, even in uncontrolled humidity environments, and serves as an alternative to storage in a dry storage cabinet. The moisture-barrier foil bag should be opened just before use. Samples stabilized for storage should be inserted along with a desiccant packet into the pouch and heat-sealed. Alternatively, store dried samples in a dry storage cabinet at room temperature (15-25°C). Unused products should be placed back in their original packaging, zip-lock-sealed and stored at room temperature in a clean, dry environment.

For optimal results when shipping samples stabilized in RNAstable tubes or 96-well plates, please utilize the moisture barrier foil bag (sample pouch) along with a desiccant packet and heat seal to insure a dry environment.

Note: Please visit www.biomatrica.com for ordering information on dry storage cabinets customized for the RNAstable product or extra sample pouches, or desiccant packs.

Applications

Sample Type:

RNAstable has been used extensively for room temperature dry storage of purified 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 along with a desiccant pack, heat-sealed 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 Sample Pouches/Desiccant Packs are available for purchase as sets of 12, catalog number 14001-007. 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 µl of water can be applied directly into an RNAstable tube or well and dried with a vacuum concentrator (e.g. using a SpeedVac). For optimal results, do not exceed 100 µg of total RNA per tube or well in a maximum volume of 100 µl water.

Sample Storage

Store dried samples in either (a) a dry storage cabinet at room temperature (15-25°C or 59-77°F) (b) a heat-sealed, moisture-barrier bag along with a silica gel desiccant packet.

RNAstable products are supplied in moisture-barrier foil bags along with a desiccant packet. Extra Sample Pouches/Desiccant Packs are sold as sets of twelve, catalog number 14001-007. Visit www.biomatrix.com for complete product listings and details.)

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.

Protocol: Sample preparation

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

Types of RNA

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

Purification Techniques

Most standard molecular biology techniques and/or commercially available kits are compatible with RNAsable 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 RNAsable.

Determining yield

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

For optimal protection in RNAsable, 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 with a vacuum concentrator (e.g. SpeedVac).

Protocol: Sample drying and storage

RNAstable protects 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. This medium is completely dissolvable and ensures total sample recovery. 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. 17 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 RNA 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 $\leq 20 \mu\text{l}$ for airdrying; do not exceed applying 100 μl water per tube or well.

- 3. Mix the sample thoroughly with gentle pipetting (avoid forming air bubbles).**
- 4. For convenient air-drying of volumes $\leq 20 \mu\text{l}$, leave the tube or 96-well plate open overnight in laminar flow hood.**

For volumes $\geq 20 \mu\text{l}$, or for accelerated drying of all, please use a vacuum concentrator (*i.e.* SpeedVac) **without heat** to ensure complete sample drying. Recommended drying times are provided in Table 1 (pg. 12).

Note: Drying should occur at ambient laboratory temperatures (15°C–25°C) (see pg. 6).

Table 1. Drying times with SpeedVac (no heat)

Sample Volume	Times
10 - 20 µl	0.5 hrs
>20 - 30 µl	1 hrs
>30 - 100 µl	1.5 hrs

- 5. For sample storage, close tube or well after sample drying and store at room temperature (15–25°C) in either a dry storage cabinet or a sealed moisture barrier foil bag.**

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

RNAstable is sensitive to temperature and humidity, therefore it is critical to maintain stored samples at room temperature (15-25°C) in an environment protected from moisture.

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 µl 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 rehydrate 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. 17) for directions on how to perform UV spectrophotometry (*i.e.* A_{260} reading) using samples stored in RNAsable.

4. Store unused rehydrated samples at 4°C or room temperature for up to 3 days. Samples can also be stored frozen (-20°C or -80°C) for longer time periods.

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

Protocol: Downstream applications

Rehydrated RNAsable 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.biomatrica.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 RNAsable is extremely effective for storage of samples at room temperature, between 15-25°C for long-term storage and temperature variations of up to 50°C. RNAsable is a dry storage technology and samples must be protected from moisture. Stabilized samples should be placed in either (a) a dry storage cabinet or (b) a heat-sealed, moisture-barrier bag along with a silica gel desiccant packet to ensure a permanently controlled environment. High humidity will hydrate RNAsable, resulting in sample degradation.

The following troubleshooting guide may be helpful in solving any problems that arise. Scientists at Biomatrix's Technical Service Department are available to answer questions about the information and protocols in this handbook or general molecular biology applications (see pg. 24 for contact information).

Situation	Comment	Suggestion
RNA is currently in TE buffer.	RNA must be in water prior to application into RNAsable.	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 RNAsable.	Place hydrated sample in laminar flow hood and allow to partially or fully dry. Use partially dried sample or rehydrate fully dried sample in correct volume.
Unused RNAsable color is faded or has a slight change in coloration.	Fading or color change of RNAsable 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 RNAsable 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 RNAsable for future reference.• Make sure sample is applied to center of well or tube containing RNAsable 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 either inside sealed moisture barrier bags or in a dry storage cabinet 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
Less volume is recovered from well than was initially added for rehydration.	Rehydration of the dissolvable matrix may cause a reduction in sample volume recovery. This is especially noticeable for smaller volumes (i.e. <20 µl)	<ul style="list-style-type: none"> • Minimal loss of recovered sample volume does not affect the stability or performance of RNA or RNAsable 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 RNAsable

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

Procedure

1. Prepare sample and blank

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

Sample: Rehydrate well or tube containing sample stored in RNAsable 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 RNAsable-only reference sample. Rehydrate an unused tube or well containing only RNAsable (*i.e.* no RNA) using the same volumes (including dilutions) as was performed for sample preparation.

2. Reference the spectrophotometer against RNAsable.

Blank the spectrophotometer using the RNAsable-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 RNAsable

Samples recovered from dry storage in RNAsable 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 μ l 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/ μ l in DEPC-treated water and 5 μ l was added to wells containing RNAsable 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 μ l of water for 15 min at room temperature and mixed gently prior to use.
3. For each QPCR reaction, 10 μ l of rehydrated sample was added to 40 μ l of QPCR master mix containing:

25 μ l 2X Master Mix without UNG (ABI)
1.25 μ l 40X Multiscribe and RNase Inhibitor Mix
1 μ l 10 μ M forward primer
1 μ l 10 μ M reverse primer
1.25 μ l 10 μ M probe
10.5 μ l water
+10 μ l rehydrated RNA sample
50 μ l 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 RNAsable is quality control tested for contamination and functional performance. All products are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

RNAsable 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. RNAsable 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 RNAsable occurs when samples are prepared and stored at room temperature and protected from moisture. Please refer to "Sample drying and storage" (pg. 9) for details on preparing and storing RNA.

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.

Literature Citation

When a procedure utilizing this product is described in a manuscript for publication, kindly refer to it as RNAsable from Biomatrix, 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.biomatrix.com, in PDF format).

Ordering Information

PRODUCT NAME	CATALOG NUMBER	DESCRIPTION
Trial Kit	93220-001	(3) Tubes of RNAsable
Tube Kit	93221-001	(25) Tubes of RNAsable
96 Well Plate	90221-001	(2) RNAsable 96-well plates
Alphanumeric Tube Plate	94231-001*	(1) 96 Tubes in 96-well footprint
2D Barcoded Tube Plate	94241-001*	(1) 96 Tubes in 96-well footprint

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 RNAsable technology, product use, or general matters.

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