

DNAstable® Plus Handbook

Preserve and store DNA samples at room temperature

Protocols for Sample Protection, Sample Recovery
and Downstream Applications

Biomātrica®
THE BIOSTABILITY COMPANY

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Introduction

Biomatrica 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 laboratories to decrease their reliance on freezers and drastically reduce shipping costs.

DNAstable *Plus* is a unique storage medium that preserves purified genomic DNA, at room temperature, in liquid format for up to one year, and as a drydown for longer storage periods. Dried DNAstable *Plus* allows for long-term stabilization of DNA samples with easy sample recovery by simply adding water.

Stabilize and store purified DNA at Room Temperature.

DNAstable® *Plus* enables you to preserve, store and ship your purified DNA samples at room temperature in liquid and dry state. It offers workflow flexibility with thermostability at even extreme temperatures and ability to handle high concentrations of DNA. Purified DNA is preserved in liquid or dry-down state. Recovery from dry matrix is achieved within minutes by simply adding water. Sample is ready for any downstream analysis without need for any additional purification step.

DNA protection is achieved during processing and storage throughout the workflow:

- 1. Liquid Stabilization (short and medium-term)**
- 2. Liquid-to-Dry (LD) Stabilization (long-term)**

Using DNAstable *Plus*, you can completely recover your dry DNA samples by simple rehydration - *just add water*. In addition, you can concentrate your sample during recovery. Samples stored in liquid format or rehydrated are ready for immediate use, without the need for further purification. Downstream applications using recovered samples include:

- Restriction enzyme analysis
- Sequencing
- PCR
- Quantitative PCR
- SNP genotyping

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

Principles and Procedures

DNAstable *Plus* is a mixture of dissolvable stabilizers that protect DNA from degradation. Based on the unique thermo-stable properties of Biomatrica's proprietary formulation, DNAstable *Plus* forms a protective seal around DNA as it dries, effectively "shrink-wrapping" the sample in a protective coating. Drying can occur at ambient temperatures with a vacuum concentrator (i.e. SpeedVac®) or through passive air drying. Stored dry at ambient temperatures, the protected DNA can be safely stored for extended time periods. Currently, Biomatrica has accelerated aging studies indicate that DNA can be safely stored for up to 10 years at room temperature. Please note that we recommend storing dry samples in either a dry storage cabinet or a heat-sealed, moisture-barrier bag. Samples can be recovered through simple rehydration and are ready for immediate use, without the need for further purification.

Storage

DNAstable *Plus* reagent can be stored at 4°C to 20°C until ready for use. The shelf-life for the reagent is indicated on the label. As an example, Best use before 04/12 corresponds to the last date of April 2012.

Extra 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 protected for Liquid-to-Dry (LD) 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).

For optimal results when shipping dry samples protected in DNAstable *Plus*, please utilize moisture barrier foil bag (sample pouch) along with a desiccant packet.

Please visit www.biomatrica.com for ordering information on dry storage cabinets customized for the DNAstable *Plus* product or extra sample pouch/desiccant packs.

Applications

Sample Type:

DNAstable *Plus* has been used for room temperature dry storage of genomic DNA.

Assay Type

DNA stored in DNAstable *Plus* is ready for immediate use in the following applications:

- SNP analysis
- Agarose-gel Electrophoresis
- PCR
- Restriction enzyme analysis
- Next-Generation Sequencing
- Quantitative PCR

Shipping:

DNAstable *Plus* provides the ideal format for transport and shipping of samples at ambient temperatures. Individual tubes or bottles can be shipped conveniently at room temperature, 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 DNAstable *Plus*. (Additional Sample Pouches/Desiccant Packs are available for purchase as sets' of 10, catalog number 14001-007. Visit www.biomatrica.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 DNA at room temperature. For optimal protection and sample recovery, DNA should be purified of any contaminating DNase activity.

Sample Application and Drying

To store DNA samples in liquid format, just add the 5X DNAstable *Plus* buffer and store at room temperature. Samples to be stored in dry format in DNAstable *Plus* must be dried completely for optimal protection and stability during room temperature storage. Sample volumes of up to 50 µl

can be applied directly to DNAstable *Plus* and dried at ambient temperatures on the lab bench or in a laminar flow hood (recommended). Larger sample volumes may require use of a vacuum concentrator for complete drying.

For optimal results, store between 1ng and 250 µg of total DNA per tube in liquid format, or up to 50ug in dry format.

Sample Storage

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

Sample Recovery

To recover samples stored in dry format in DNAstable *Plus*, just add water. Samples are 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 HCl, 1 mM EDTA), PCR reaction buffers, and restriction enzyme buffers are also compatible with the recovery of samples from DNAstable *Plus*.

Protocol: Sample preparation

This protocol is designed for the preparation of purified genomic DNA for storage in DNAstable *Plus*.

Purification Techniques

Most standard molecular biology techniques and/or commercially available kits are compatible with DNAstable *Plus* storage. For optimal results, DNA samples should be free of any contaminating DNase activity.

Purified DNA that is DNase-free and resuspended in water or a low salt buffer can be used directly with DNAstable *Plus*.

Determining yield

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

For optimal results, store between 1 ng and 250 µg of total DNA per tube in liquid format, or up to 50 µg in dry format. .

Protocol: Sample drying and storage

DNAstable *Plus* preserves DNA samples at room temperature. Each tube or bottle contains DNAstable *Plus* provided as 5X buffer solution which protects nanogram to microgram amounts of DNA. The sample can then be stored in liquid for up to a year at room temperature. For longer term storage, the sample must be completely dried for maximum protection and stability for storage at ambient temperatures.

Note: In the event you want to determine the DNA concentration at a later date using a spectrophotometer, keep an unused amount of DNAstable *Plus* to generate a blank. See **Appendix A**, page 14.

Procedure

1. Add 25% (vol:vol) of the reagent to the DNA sample ($\leq 250\mu\text{g}$), stored in water or any aqueous buffer (e.g., 25 μl of DNAstable *Plus* to 100 μl of DNA).
2. Mix the sample thoroughly with gentle pipetting. Avoid forming air bubbles.
3. Store at room temperature for up to a year, or proceed to 4.
4. Dry the uncovered sample completely at room temperature (15–25°C). We recommend using a laminar flow hood or drying under a vacuum concentrator to ensure complete drying. Recommended drying times are given in Tables 1 and 2.

Note: Drying should occur at ambient laboratory temperatures (15°C–25°C). Exposure to moisture for extended periods of time will reduce product performance and sample protection. Climate-controlled laboratory environments and buildings are normally maintained at 40–50% relative humidity levels. We recommend drying under a vacuum concentrator if conditions exceed these parameters.

Table 1. Minimal Drying Times in a Laminar Flow Hood.*

Volume (µl)	Drying Times (hrs) Tubes	Drying Times (hrs) (96-well plate)	Drying Times (hrs) (384-well plate)
5	4	4	8
6-10	6	6	12
11-20	12	8	24
21-50	28	18	48
51-100	56	24	68
101-125	72	24	78

***Drying time may vary dependent on the humidity level in the laboratory. Recommended drying times were determined at 50% relative humidity (RH); (typical HVAC controlled facilities have 40-50% RH). Volume and Drying times refer to the mixture of sample and DNAstable *Plus* reagent.**

Longer drying times are preferable to ensure complete drying. Completely dried samples should NOT feel sticky or tacky when tapped with a sterile pipette tip.

Table 2. Minimal Drying Times in a Vacuum Concentrator at Low Temperature (25-30°C)**

Volume (µl)	Drying Times (Min) Tubes	Drying Times (Min) (96-well plate)	Drying Times (Min) (384-well plate)
5	10	15	80
6-10	15	15	120
11-20	30	30	180
21-50	45	90	360
51-125	60	150	--
125-150	75	180	--

**** Drying times may vary depending on model and condition of vacuum concentrator used. Volume and Drying times refer to the mixture of sample and DNAstable *Plus* reagent.**

Note: Do not exceed 50 µg of DNA per well or tube to ensure optimal protection.

5. Cover samples after drying and store at room temperature, protected from moisture.

After complete sample drying, plates should be re-sealed with aluminum foil seals. Tubes can be closed using the cap supplied with the tube.

Protocol: Drydown Sample recovery

DNA stored dry in DNAstable *Plus* 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 water or aqueous buffer directly to the dried sample in DNAstable *Plus* tube or well, to the same volume as dried down sample.

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

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

Note: Samples can be easily concentrated at this step if the amount of water or buffer used to rehydrate a known amount of dried DNA is smaller than the original sample. However, downstream applications might be affected by this concentration.

2. Incubate at room temperature for 15 minutes to allow complete rehydration.

3. Mix the sample by gently pipetting up and down to resuspend the sample. Avoid forming bubbles while pipetting.

The rehydrated sample is now ready for use in downstream applications. Real-time PCR analysis using SYBR® Green or sequence-specific probes should be performed using the guidelines as provided in **Appendix B**, page 17.

To determine DNA concentration and recovery yield, see **Appendix A** on page 14 for directions on how to perform UV spectrophotometry (i.e. A_{260} reading) using samples stored in DNAstable *Plus*. Spectrophotometer must be zeroed against a DNAstable *Plus* blank.

Store unused rehydrated samples at 4°C or room temperature for medium term storage (up to 1 month).

Rehydrated sample contain DNAstable *Plus* and can be re-dried without lost of efficient sample stabilization. We do not recommend repeating this process more than 5 times.

Protocol: Downstream applications

Rehydrated DNAstable *Plus* 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 page 7 for complete list of suitable applications. For further results and examples, refer to www.biomatrica.com under *Application Notes*.

Real-time PCR analysis using SYBR® Green or sequence-specific probes should be performed using the guidelines as provided in **Appendix B**, page 15.

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

Troubleshooting Guide

Dry storage of DNA in DNAsable *Plus* 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 and protected from moisture). Stabilized samples should be placed in either (a) a dry storage cabinet at room temperature (15-25°C) or (b) a heat-sealed, moisture-barrier bag to ensure a permanently controlled environment. Significant moisture content in the air will hydrate DNAsable *Plus*, resulting in sample degradation.

The following troubleshooting guide may be helpful in solving any problems that arise. Scientists at Biomatrica'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
DNases in sample	DNA to be stored in DNAsable <i>Plus</i> must be free of contaminating DNase activity for optimal protection.	Ensure that samples are purified so that they are DNase-free before applying to DNAsable <i>Plus</i> .
Low amount of DNA in initial sample	Before applying the sample, measure the concentration and record the amount of DNA added.	Identical samples can be rehydrated separately.
Sample not properly applied	Sample must be diluted into 5X DNAsable <i>Plus</i> buffer, to ensure stabilization.	Add 4 parts of DNA solution to 1 part of DNAsable <i>Plus</i> buffer and store at room temperature. For long term storage, dry sample and store at room temperature.
Too much buffer used for rehydration	Use of large volumes (>100 µl) for rehydration may cause overflow of wells and cross-contamination between wells.	Samples can be rehydrated in the recommended volumes and then transferred to a larger vessel and brought up to a larger volume.
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 DNA or DNAsable <i>Plus</i> in downstream applications.A 10% loss in volume can be included for adjustment (e.g. add 110 µl of water to ensure recovery of 100 µl of rehydrated sample).

Appendix A: UV spectrophotometry with DNAstable *Plus*

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

Procedure

1. Prepare sample and blank

The UV spectrophotometer must be calibrated against an aliquot of DNAstable *Plus* for accurate A_{260} readings.

Sample: Rehydrate well or tube containing sample stored in DNAstable *Plus* 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: Calibrate the spectrophotometer against an aliquot of DNAstable *Plus* for use as the reference sample. **Reference the spectrophotometer against DNAstable *Plus*.**

Calibrate the spectrophotometer using the reference sample.

2. Determine A_{260} reading of sample.

3. Calculate concentration of sample.

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

For double-stranded nucleic acid

$$\text{Conc. } (\mu\text{g/ml}) = (A_{260} \text{ reading}) (50 \mu\text{g/ml}) \text{ (dilution factor)}$$

For single-stranded nucleic acid

$$\text{Conc. } (\mu\text{g/ml}) = (A_{260} \text{ reading}) (38 \mu\text{g/ml}) \text{ (dilution factor)}$$

Appendix B: Quantitative PCR using samples stored in DNAstable *Plus*

To quantify samples using Picogreen

Add DNAstable *Plus* directly to the Picogreen standards. For example, if you recovered your DNA sample stored in DNAstable *Plus* in 20 μ l and you are quantifying 2 μ l of sample, add 2 μ l of DNAstable *Plus* to 18 μ l of Picogreen standards.

Using TaqMan®

A 1:5 final dilution of sample:PCR Master Mix total final volume is recommended. Samples should be rehydrated in a volume appropriate for up to 50 μ l QPCR reaction (total final volume) prior to following manufacturer's instructions.

Example: TaqMan® PCR Master Mix Reagents Kit

1. Human genomic DNA (Novagen; Madison, WI) was diluted to 0.2 ng/ μ l in water or 1X DNAstable *Plus* matrix and 10 μ l dried down in a safety hood.
2. Samples were later rehydrated with 10 μ l of water as described in "Recovering DNAstable *Plus* Samples" steps 1-5 above.
3. For each QPCR reaction, the entire 10 μ l of rehydrated sample was added to 40 μ l of QPCR master mix containing:

25.00 μ l TaqMan Universal PCR Master Mix (Applied Biosystems)

- 1.25 μ l Probe
- 1.00 μ l 10 μ M forward primer
- 1.00 μ l 10 μ M reverse primer
- 11.75 μ l Water
- 10.00 μ l Rehydrated DNA sample
- 50.00 μ l Total Final Volume**

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

Initial step:

50°C for 2 min

95°C for 10 min

Remaining steps:

40 cycles each

95°C for 15 sec

60°C for 1 min

Results from Example: TaqMan® PCR Master Mix Reagents Kit

The concentration of DNA as determined by QPCR was found to be equivalent between test and control samples ($\pm 10\%$). A slope of -3.1 to -3.6 and an R^2 value of 0.99 are considered acceptable.

SYBR® Green Technology

A dilution of sample to PCR total volume is recommended for DNAstable *Plus* samples used in QPCR with SYBR Green reagents. Up to 30% of the QPCR reaction can be composed of the 1X DNAstable *Plus* matrix.

Example SYBR® Green reaction

1. Human genomic DNA (Novagen; Madison, WI) was diluted to 0.2 ng/ μ l in 20 μ l of water or 1X DNAstable *Plus* formulation and then dried down in a safety hood.
2. Samples were rehydrated with 20 μ l of water as described in "Recovering DNAstable *Plus* Samples" steps 1-5 above.
3. For each QPCR reaction, 7.5 μ l of rehydrated sample was added to 17.5 μ l of QPCR master mix following manufacturer's instructions, to a final QPCR reaction volume of 25 μ l.
4. Use thermal cycling reaction conditions as recommended by the manufacturer.

Quality Control

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

Product Use Limitations

DNAstable *Plus* technology is specifically designed for the stabilization of purified DNA samples. No claim or representation is intended for their use on any other biological materials. DNAstable *Plus* 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 DNA in DNAstable *Plus* occurs when samples are prepared and stored at room temperature and, for dry storage, in a relative humidity environment $\leq 40\%$. Please refer to "Sample drying and storage" (pg. 8) for details on preparing and storing DNA under relative humidity conditions exceeding 40%.

Warranty and Satisfaction Guarantee

Biomatrica, 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, Biomatrica will replace it free of charge or refund the purchase price, at your request. Biomatrica reserves the right to change, alter, or modify any product to enhance its performance and design. Contact Biomatrica if a product does not meet your expectations.

Warranties of merchantability or fitness for a particular purpose are expressly disclaimed. Biomatrica's liability shall not exceed the purchase price of the product. Biomatrica 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 Biomatrica's full limited warranty statement is available at www.biomatrica.com or upon request.

Literature Citation

When a procedure utilizing this product is described in a manuscript for publication, kindly refer to it as DNAstable *Plus* 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).

Ordering Information

PRODUCT NAME	CATALOG NUMBER	DESCRIPTION
DNAstable <i>Plus</i> 2ml	53091-016	(1) 2ml tube of liquid DNAstable <i>Plus</i> for liquid and Liquid-to-Dry (LD) storage of DNA
DNAstable <i>Plus</i> 10ml	52091-026	(1) 10ml bottle of liquid DNAstable <i>Plus</i> for liquid and Liquid-to-Dry (LD) storage of DNA

NOTES

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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 DNAsable Plus technology, product use, or general matters.

Technical Service Department:

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