DNAstable® Blood Handbook

Preserve DNA in biological samples at room temperature

For room temperature shipping, storage and archiving of blood and buffy coat for DNA preservation

Biomatrica®
THE BIOSTABILITY COMPANY

Item No. 5001-0612 / Rev. 06/2012

Contents

Kit Contents

DNAstable Blood	Tubes (50)	48-well Plate (1)	48-well Plates (10)	96-well Plates (1)	96-well Plates (10)
Catalog no.	93027-027	57021-047	57022-357	90621-026	90622-026
Number of preps	50	48	10 x 48	96	10 x 96
DNAstable Blood Tubes	50	--	--	--	--
DNAstable Blood Tube Carrier	1	--	--	--	--
DNAstable Blood 48-well plates	--	1	10	--	--
DNAstable Blood 96-well plates	--	--	--	1	10
AirPore Tape Sheets	--	1	10	1	10
Desiccants	5	1	10	1	10
Moisture Barrier Bags	5	1	10	1	10
Protocol Card	1	1	1	1	1

Storage

DNAstable Blood matrix is a technology for safe, stable, and convenient storage of whole blood and buffy coat at room temperature (15–25°C). DNAstable Blood tubes or plates should be stored in their original unopened packaging before use.

DNAstable Blood tubes and plates are supplied with moisture barrier foil bags. For optimal product assurance DNAstable Blood plates and tubes need to be stored in a low relative humidity environment. It is recommended to store plates or tubes in a heat-sealed moisture barrier bag with the desiccant that is provided. For extended storage times (>6 months), moisture barrier bags should be heat-sealed. Alternatively, tubes or plates may be stored in a dry storage cabinet with relative humidity below 40% (see “Equipment and Reagents to Be Supplied by User”, page 7). Storage of opened DNAstable Blood tubes and plates at relative humidity above 40% for extended periods of time will reduce product performance and sample protection.

Quality Control

In accordance with BIOMATRICA’s Quality Management System, each lot of DNAstable Blood tube and plates is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

DNAstable Blood tubes and plates are intended for research use.

Product Warranty and Satisfaction Guarantee

BIOMATRICA guarantees the performance of all products in the manner described in the product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, BIOMATRICA will replace it free of charge. We reserve the right to change, alter, or modify any product to enhance its performance and design. A copy of BIOMATRICA terms and conditions can be obtained on request. If you have questions about product specifications or performance, please call Biomatrica Technical Services or your local distributor (visit www.biomatrica.com).

Technical Assistance

At BIOMATRICA, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of BIOMATRICA products. If you have any questions or experience any difficulties regarding DNAstable Blood, or BIOMATRICA products in general, please do not hesitate to contact us.

BIOMATRICA customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at BIOMATRICA. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact us at contact@biomatrica.com

Introduction

DNAstable® Blood provides innovative technology for biological sample storage at room temperature. Stabilizing blood at room temperature saves on refrigeration and shipping costs as well as enables easy transportation and storage.

DNAstable technology was designed by combining extremophile biology and synthetic chemistry to create a novel dissolvable matrix optimally formulated for long-term ambient temperature storage and shipping of whole blood and buffy coat.

Rehydrated blood samples are ready for immediate DNA purification using standard DNA purification techniques such as the Qiagen QIAamp DNA Blood Mini Kit and organic extraction techniques. The purified DNA is suitable for any downstream applications such as PCR, quantitative PCR, and microarray analysis.

Blood and buffy coat samples stored dry in the DNAstable Blood matrix are ready for convenient shipping at ambient temperatures, even over extended transit times.

After applying and subsequent drying of blood samples with DNAstable® Blood, DNA is stabilized *in situ* for years at room temperature (15–25°C). DNAstable® Blood can be easily transported, archived, or processed immediately.

Principle and Procedure

The DNAstable Blood procedure consists of two parts: sample collection and nucleic acid purification. Blood and buffy coat samples are directly applied to the DNAstable Blood matrix.

The DNAstable Blood matrix is a mixture of dissolvable compounds that stabilizes DNA at room temperature. The matrix formulation is based upon the natural principles of anhydrobiosis. Anhydrobiosis, meaning “life without water”, is a biological mechanism employed by some multicellular organisms that enables their survival in a dry state for periods over 100 years.* During these extended dry periods, proteins, DNA, membranes, and cellular systems are protected and can be revived by rehydration.

The DNAstable Blood matrix 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 no need for special equipment. Stored dry at ambient temperatures, the protected blood or buffy coat can be safely stored for extended time periods.

Blood and buffy coat can be recovered from the DNAstable matrix through a simple rehydration protocol and is ready for immediate DNA purification.

DNA can be purified from the rehydrated blood or buffy coat using a variety of different kits for manual or automated DNA. The QIAamp® DNA Blood Mini and QIAamp® DNA Mini Kit provide easy manual protocols. Both protocols are fully automatable on the QIAcube®. Automation on EZ1® workstations provides purification of DNA from 1–6 samples per run using the EZ1 DNA Blood Kits. For medium throughput, the QIAsymphony® SP module can process 1–48 samples per run using the QIAsymphony DNA Mini or the QIAsymphony DNA Midi Kit.

* Crowe, J.H., Carpenter, J.F., and Crowe, L.M. (1998). The role of vitrification in anhydrobiosis. *Annu. Rev. Physiol.* 60, 73.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDS's), available from the product supplier.

For drying of samples on DNAstable Blood tubes or plates

- Pipets and pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended).
- Recommended: Laminar flow hood, ventilated incubator set to 40°C, or vacuum concentrator (SpeedVac or Vacufuge).
- Shaker with an adapter for a microtiterplate.
- Heat sealer.
- Optional: Dry storage cabinet with relative humidity below 40% (e.g., dry storage cabinets from Biomatrica, www.biomatrica.com/drystoragecabinets.php).

For purification of DNA from DNAstable Blood tubes or plates

- Pipets and pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended).
- Shaker with an adapter for a microtiter plate or microtiter plate mixer.
- QIAamp® DNA Blood Mini Kit (cat. no. 51104), QIAamp DNA Mini Kit (cat. No. 51304), EZ1 DNA Blood 200µl Kit (cat. no. 951034), or QIAsymphony DNA Mini Kit (cat. no. 931236)*

Protocol for Blood or Buffy Coat Storage (48-well Plates & Tubes)

Sample Drying and Storage

The DNAstable Blood matrix is formulated so that, upon application of liquid samples, the matrix dissolves and forms a protective coating. The sample must then be completely dried for maximum protection and stability during storage at ambient temperatures.

Important points before starting

- Buffy coat may vary considerably in leukocyte concentration depending upon the number of nucleated cells in the original whole blood sample and the efficiency of leukocyte harvesting during the buffy coat preparation. Efficiency of buffy coat enrichment depends on the sample preparation procedure used and on the accuracy used when extracting the buffy coat layer.
- For optimal protection in DNAstable matrix, do not apply more than a total of 200 µl of blood or buffy coat per well or tube. Samples should be dried in a laminar flow hood or in in a ventilated incubator set to 40°C. At an elevated relative humidity >40%, drying time may be significantly longer. Samples applied to wells or tubes containing DNAstable Blood matrix must be dried completely for optimal storage at room temperature.
- Fresh or frozen whole blood can be used collected with EDTA, sodium citrate, or heparin as anticoagulants.
- Always wear gloves when handling DNAstable Blood tubes or plates to avoid contamination.

Procedure

1. **Remove the cover or cap and gently apply 50–200 µl of whole blood or buffy coat into the center of the vessel containing DNAstable Blood matrix.**

   DNAstable Blood matrix is supplied as a coating on the bottom of each well or tube.

   Note: If storing 50–100 µl of sample in a well of a DNAstable Blood 48-well plate, first add 50 µl nuclease-free water and then the sample to the well.

2. **Mix the sample by placing the DNAstable Blood 48-well plate or tube (placed in the provided carrier plate) on a microtiter plate shaker, and shake for 15 min at 600-800 rpm.**

   Note: To avoid cross-contamination of samples, do not shake at higher speeds.

3. **Dry the uncovered sample completely under laminar flow hood at room temperature (15–25°C), or by placing in a ventilated incubator or oven set to 40°C.**

   Drying times are dependent on the sample volume and the drying conditions. Approximate drying times are given in Tables 1 and 2 on page 9.

To ensure complete drying, we recommend drying the samples overnight (i.e., >16 h) using a laminar flow hood at room temperature or a ventilated incubator set to 40°C.

Table 1. Approximate Drying times in laminar flow hood

Sample volume (µl)	Approximate drying time (h)	
	Tubes	**48-well plates**
50	4	-
100	6	7
200	8	14

Table 2. Approximate Drying times in a ventilated incubator at 40°C

Sample volume (µl)	Approximate drying time (h)	
	Tubes	**48-well plates**
50	3	-
100	5	4
200	6	7

Note: Drying times in Table 1 and Table 2 are only estimations. Depending on the relative humidity drying time may vary significantly. Drying should occur at 15–40°C with relative humidity below 40%. Please be aware that drying in a non-ventilated incubator leads to elevated relative humidty.

4. Cover the samples after drying.

After sample drying, plates should be covered with an AirPore Tape Sheet (provided). DNAstable Blood tubes can be closed using the caps supplied with the tubes.

5. Storage

Tubes or plates must be stored in a humidity controlled environment such as:

- Automated store
- Storage cabinets with desiccant or humidity control
- Moisture barrier bags

If the moisture barrier bag is selected, place the samples including a desiccant in a heat-sealed moisture barrier bag.

Remove a desiccant packet from the protective bag and place it together with the sample in the moisture barrier bag. Make sure that the color indicator beads of the desiccants are dark blue (pink beads indicate exposure to moisture; in this case, the desiccants should not be used). Moisture laden desiccant packets can be regenerated by placing them in a ~110°C oven until they turn dark blue.

For extended storage times (>6 months), moisture barrier bags should be heat-sealed.

Sample Recovery

Genomic DNA from whole blood or buffy coat stored in DNAstable Blood can be recovered by following the protocol below. After rehydration the DNA can be purified using standard DNA purification protocols for whole blood.

Procedure

1. Add 300 µl of DNAse free water directly to the dried sample in a DNAstable Blood tube or well.
2. Place the plate or the tubes (placed in the supplied tube carrier plate) on a thermal shaker, and incubate at room temperature with continuous shaking at 1000-1200 rpm until the sample is rehydrated.

   Rehydration time is dependent on the original sample volume, the storage time and the temperature, and may vary from 1 to 2 hours until the sample returns to liquid form. To avoid cross-contamination of samples, do not shake at higher speeds.
3. Transfer the rehydrated sample to a clean microfuge tube and proceed with the manufacturers' recommendation for digestion and extraction of the DNA (i.e. the blood or buffy coat purification protocol in the QIAamp® mini purification kit from Qiagen.)

Protocol for Blood or Buffy Coat Storage (96-well Plate)

Sample Drying and Storage

The DNAstable Blood matrix is formulated so that, upon application of liquid samples, the matrix dissolves and forms a protective coating. The sample must then be completely dried for maximum protection and stability during storage at ambient temperatures.

Important points before starting

- For optimal protection in DNAstable matrix, do not apply more than 25µl of blood or buffy coat per well. Samples should be dried in a laminar flow hood or in a vacuum concentrator (SpeedVac or Vacufuge). At an elevated relative humidity >40%, drying time may be significantly longer. Samples applied to wells containing DNAstable Blood matrix must be dried completely for optimal storage at room temperature.
- Fresh or frozen whole blood can be used collected with EDTA, sodium citrate, or heparin as anticoagulants.
- Buffy coat may vary considerably in leukocyte concentration depending upon the number of nucleated cells in the original whole blood sample and the efficiency of leukocyte harvesting during the buffy coat preparation. Efficiency of buffy coat enrichment depends on the sample preparation procedure used and on the accuracy used when extracting the buffy coat layer.
- Always wear gloves when handling DNAstable Blood tubes or plates to avoid contamination.

Procedure

1. **Remove the cover or cap and gently apply 1–25 µl of whole blood or buffy coat into the center of the vessel containing DNAstable Blood matrix.**

   DNAstable® Blood matrix is supplied as a coating on the bottom of each well or tube.

   Note: If storing 10–15 µl of blood or buffy coat in a well of a DNAstable Blood 96-well plate, first add 10 µl of nuclease-free water and then the blood sample to the well.

2. **Mix the sample by placing the DNAstable Blood 96-well plate on a microtiter plate shaker, and shake for 15 min at 500 rpm.**

Note: To avoid cross-contamination of samples, do not shake at higher speeds.

3. Dry the uncovered sample completely under **laminar flow hood, for 6h** at room temperature (15–25°C). Alternatively, place an Airpore tape sheet

(provided) over the plate and dry in a vacuum concentrator **(SpeedVac or Vacufuge) at room temperature for 4h** (temperatures up to 45°C can be used without effect on DNA quality).

4. Drying times are dependent on the blood or buffy coat volume and the drying conditions. Depending on the relative humidity, drying time may vary significantly. Drying should occur at 15–40°C with relative humidity below 40%. To ensure complete drying, we recommend drying the samples overnight (i.e., >16 h) using a laminar flow hood at room temperature or a vacuum concentrator (SpeedVac or Vacufuge). After sample drying, plates should be covered with an AirPore tape sheet (provided).

5. **Storage**

   Plates must be stored in a humidity controlled environment such as:

   - Automated store
   - Storage cabinets with desiccant or humidity control
   - Moisture barrier bags

If the moisture barrier bag is selected, place the samples including a desiccant in a heat-sealed moisture barrier bag.

Remove a desiccant packet from the protective bag and place it together with the sample in the moisture barrier bag. Make sure that the color indicator beads of the desiccants are dark blue (pink beads indicate exposure to moisture; in this case, the desiccants should not be used). Moisture laden desiccant packets can be regenerated by placing them in a ~110°C oven until they turn dark blue.

For extended storage times (>6 months), moisture barrier bags should be heat-sealed.

Sample Recovery

Genomic DNA from whole blood or buffy coat stored in DNAstable Blood can be recovered by following the protocol below. After rehydration the DNA can be purified using standard DNA purification protocols for whole blood or buffy coat.

Procedure

6. Add 100 µl of DNAse free water directly to the dried blood in a DNAstable Blood well.
7. Place the plate on a microtiter plate shaker, and incubate at room temperature with continuous shaking at 500 rpm until the sample is rehydrated (15-30 minutes).

   To avoid cross-contamination of samples, do not shake at higher speeds.
8. Transfer the rehydrated sample to a clean microfuge tube and proceed with the manufacturers' recommendation for digestion and extraction of the DNA (i.e. the blood purification protocol in the QIAamp® mini purification kit from Qiagen).

Appendix A: Determination of concentration, yield, and purity

DNA yield is determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. Absorbance readings at 260 nm should fall between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly: for example, an eluate containing 25–50 ng DNA/µl (A260 = 0.5–1.0) should not be diluted with more than 4 volumes of buffer. Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer. Measure the absorbance at 260 and 280 nm, or scan absorbance from 220–320 nm (a scan will show if there are other factors affecting absorbance at 260 nm). Both DNA and RNA are measured with a spectrophotometer; to measure only DNA, a fluorimeter must be used.

Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an A260/A280 ratio of 1.7–1.9.

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 DNAstable technology, product use, or general matters.

Technical Service Department:
Phone: USA (866) DRY-MTRX or (866) 379-6879
Web: www.biomatrica.com
Email: contact@biomatrica.com

Biomātrica®
THE BIOSTABILITY COMPANY

5627 Oberlin Drive, Suite 120
San Diego, CA 92121