

DNAstable[®] Blood LD

Handbook

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

Biomātrica[®]
THE BIOSTABILITY COMPANY

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Product Formats

Catalog number	Volume (mL)	Description
52001-027	DNASTable Blood LD, 10 mL	10 mL screw cap bottle
52001-047	DNASTable Blood LD, 100 mL	100 mL screw cap bottle

Storage

DNASTable Blood LD is a technology for safe, stable, and convenient storage of whole blood and buffy coat at room temperature (15 - 25°C). DNASTable Blood LD bottles should be stored at 4°C in their original unopened packaging before use.

Quality Control

In accordance with Biomatrix's Quality Management System, each lot of DNASTable Blood LD is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

DNASTable Blood LD is intended for research use only.

Product Warranty and Satisfaction Guarantee

Biomatrix 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, Biomatrix 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 Biomatrix terms and conditions can be obtained on request. If you have questions about product specifications or performance, please call Biomatrix Technical Services or your local distributor (visit www.biomatrix.com).

Technical Assistance

At Biomatrix, 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 Biomatrix products. If you have any questions or experience any difficulties regarding DNASTable Blood LD, or Biomatrix products in general, please do not hesitate to contact us.

Biomatrix 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 Biomatrix. 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@biomatrix.com

Introduction

DNASTable[®] Blood LD 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 QIAamp[®] DNA Blood Mini Kit (QIAGEN) 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 LD are ready for convenient shipping at ambient temperatures, even over extended transit times.

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

Principle and Procedure

The DNASTable Blood LD is a mixture of dissolvable compounds that stabilizes DNA at room temperature. The 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 LD 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.

The DNASTable Blood LD procedure consists of two parts: sample collection and nucleic acid purification. The DNASTable Blood LD is mixed with whole blood or buffy coat specimen at a 1:1 volumetric ratio followed by aliquoting the LD-specimen mixture into a variety of plates or tubes. The plates or tubes are then be dried by a vacuum concentrator, laminar flow hood, or ventilated incubator. The following table summarizes the minimal drying time for tubes and 96-well plates in a SpeedVac at 25 - 30°C.

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

Minimal drying time in a SpeedVac at 25-30°C

Sample volume (µL)	Drying times (min)* Tube	Drying times (min)* 96-well plate
5	10	15
6-10	15	15
11-20	30	30
21-50	45	90
51-125	60	150
125-150	75	180

* Drying times may vary depending on model and condition of SpeedVac, vacuum pump, temperature, air humidity, types of samples, sample volume, types of tubes and plates.

The dried blood and buffy coat can be recovered through a rehydration process, and is then 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 purification. The QIAamp® DNA Blood Mini Kit provides an easy manual protocol. The protocol is fully automatable on the QIAcube® instrument. Automation on EZ1® workstations provides purification of DNA from 1 - 6 samples per run using 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.

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), available from the product supplier.

For drying of samples in plates or tubes:

- Pipettes and pipette tips (pipette tips with aerosol barriers for preventing cross-contamination are recommended)
- Recommended: Vacuum concentrator (SpeedVac or Vacufuge), laminar flow hood, or ventilated incubator
- Shaker with an adapter for plates or tubes
- Heat sealer
- Optional: Dry storage cabinet with relative humidity below 40% (e.g., dry storage cabinets from Biomatrix, www.biomatrix.com/drystoragecabinets.php)

For purification of DNA from whole blood or buffy coat stored with DNASTable Blood LD:

- Pipettes and pipette tips (pipette tips with aerosol barriers for preventing cross-contamination are recommended).

- Shaker with an adapter for plates or tubes.
- QIAGEN kits: 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 Whole Blood Storage (48-Well Plate)

Sample Drying and Storage

The DNASTable Blood LD forms a protective coating upon addition to liquid samples. The LD-specimen mixture must then be completely dried for maximum protection and stability during storage at ambient temperatures.

Important points before starting

- The DNASTable Blood LD-specimen mixture should be dried completely in a vacuum concentrator (SpeedVac or Vacufuge) for optimal storage at room temperature. At an elevated relative humidity >40%, drying time may be significantly longer.
- Fresh or frozen whole blood can be collected with EDTA, sodium citrate or heparin as anticoagulants.
- Always wear gloves when handling DNASTable Blood LD, blood samples, tubes and plates to avoid contamination.

Procedure

1. Mix DNASTable Blood LD and the whole blood specimen at a 1:1 volumetric ratio.

Note: We recommend adding a certain volume of DNASTable Blood LD into a well first, followed by adding the same volume of whole blood.

2. Aliquot 150 µL of the LD-specimen mixture per well into a 48-well plate.
3. Place the plate on a plate shaker with a plate adapter and shake for 10 minutes at 250 rpm.
4. Dry the samples in a vacuum concentrator (SpeedVac or Vacufuge) at room temperature (25°C) for 2 hours. The samples must be dried to completion. A basic test for sufficient sample dryness is to poke a sterile pipette mix into the matrix; if the sample adheres to the end of the pipette tip then the sample requires more drying time.

Note: Drying times are dependent on the sample volume and other drying conditions. Drying time may vary significantly depending on the relative humidity. Drying should occur at 15 - 40°C with relative humidity below 40%.

5. Seal the plate with ThermoSeal Airpore Tape sheets. Place each plate in a foil moisture barrier bag containing a desiccant pack.
6. The plate must be stored in a humidity controlled environment such as:
 - Automated store
 - Storage cabinets with desiccant or humidity control
 - Moisture barrier bags

Place each plate including a desiccant into a heat-sealed 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 stored with DNASTable Blood LD can be recovered by the following protocol. After rehydration, the DNA can be purified using standard DNA purification protocols for whole blood.

Procedure

1. Add 200 µL of DNase free, sterile, deionized water to each well containing a dried blood sample.
2. Place the plate on a plate shaker with a plate adapter, and incubate at room temperature with continuous shaking at 250 rpm until the samples are completely rehydrated (up to 2 hours). To avoid cross-contamination of samples, do not shake at higher speeds.
3. Transfer each rehydrated sample to a clean 1.7 mL microfuge tube and process DNA purification with QIAamp[®] DNA Blood Mini Kit (Cat No. 51304, QIAGEN).

Protocol for Whole Blood Storage (Micronic 1.4mL U-Bottom Tubes)

Sample Drying and Storage

The DNASTable Blood LD forms a protective coating upon mixture with liquid blood samples. The LD-specimen mixture must then be completely dried for maximum protection and stability during storage at ambient temperatures.

Important points before starting:

- The DNASTable Blood LD-specimen mixture should be dried completely in a vacuum concentrator (SpeedVac or Vacufuge) for optimal storage at room temperature. At an elevated relative humidity >40%, drying time may be significantly longer.
- Fresh or frozen whole blood can be collected with EDTA, sodium citrate or heparin as anticoagulants.
- Always wear gloves when handling DNASTable Blood LD, blood samples, tubes and plates to avoid contamination.
- This protocol is designed for drying blood samples in DNASTable Blood LD using Micronic 1.4mL U-bottom 2D bar-coded tubes and four Micronic plates (96 tubes per plate).

Procedure

1. Remove the caps from the Micronic 1.4mL U-bottom 2D bar-coded tubes.
2. The volumetric ratio of DNASTable Blood LD to the whole blood specimen should be 1:1. First, pipette 500µL of DNASTable Blood LD into each tube; then pipette 500µL of whole blood into the same tubes containing DNASTable Blood LD.
Note: We recommend transferring the 500µL of DNASTable Blood LD into the tube before transferring the 500µL of whole blood into the same tube.
3. Cover the tubes with Aeraseal Breathable Sealing Films (Excel Scientific, Cat No. BS25). This prevents cross contamination of the samples.
4. Weigh the plates to ensure that they are balanced before placing them into a SpeedVac (Savant SC250EXPA-115).
5. Place four plates into the SpeedVac and dry the samples at 55°C for 7 hours. The samples must be dried to completion.
6. Remove the plates from the SpeedVac. Allow the samples to equilibrate to room temperature for 30 minutes. Remove the sealing films from the tubes and place caps back onto the tubes.
7. The plates must be stored in a humidity controlled environment such as:
 - Automated store
 - Storage cabinets with desiccant or humidity control
 - Moisture barrier bags

Place each plate including a desiccant into a heat-sealed 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 stored with DNAstable Blood LD 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. Remove the caps from the tubes containing dried blood samples. Add 500 µL of DNase free, sterile, deionized water into each tube. Place caps back onto the tubes.
2. Place the plate on a plate shaker with a plate adapter. Shake the plate at 700 rpm at room temperature for 2 hours. Vortex the tubes intermittently every 30 minutes until the samples are dissolved.
3. Transfer each rehydrated sample to a clean 1.7 mL microfuge tube and process DNA purification with QIAamp[®] DNA Blood Mini Kit (Cat No. 51304, QIAGEN).

Appendix: Determination of DNA 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/ μ L ($A_{260} = 0.5\text{--}1.0$) 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, use Quant-iT™ PicoGreen® dsDNA Assay Kit (Cat No. P11496, Life Technologies) or a fluorimeter.

Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an A_{260}/A_{280} ratio of 1.7 - 1.9.

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Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the DNASTable® Blood LD to the following terms:

1. The DNASTable® Blood LD may be used solely in accordance with the *DNASTable Blood LD Handbook*. Biomatrixa grants no license under any of its intellectual property to use or incorporate DNASTable Blood LD with any components not included within DNASTable® Blood LD except as described in the *DNASTable Blood LD Handbook*.
2. Other than expressly stated licenses, Biomatrixa makes no warranty that DNASTable Blood LD and/or its use(s) do not infringe the rights of third-parties.
3. DNASTable Blood LD is licensed for one-time use and may not be reused, refurbished, or resold.
4. Biomatrixa specifically disclaims any other licenses, expressed or implied other than those expressly stated.

Notes

Notes

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.

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