

DNAgard[®] Blood Tube User Manual

For the collection, shipping and storage of whole
blood for DNA preservation

Cat. No: 53561-038 (50 tubes)

Cat. No: 53562-048 (100 tubes)

For Research Use Only
Not for use in diagnostic procedures

Biomātrica[®]

Contents

Introduction	3
Product Summary	3
Summary and Explanation	3
Principle of Procedure	4
Safety and Warnings	4
Specimen Collection and Preparation for Analysis	4
DNA recovery from DNAgard[®] Blood Tubes	9
Appendices	10
Frequently Asked Questions	13
Notes	15
Technical Assistance	16

Introduction

DNAgard[®] Blood Tube is a plastic, sterile, evacuated tube, pre-filled with a stabilization reagent to preserve DNA in whole blood prior to isolation of genomic DNA. Purification of genomic DNA from blood stabilized in DNAgard[®] Blood Tubes is best achieved by silica-membrane-based column technologies. In addition, we describe purification using salting out and magnetic bead technologies with minor modifications. The use of this tube is restricted to Research Use Only, and not for diagnostic procedures and patient management. Neither the clinical utility nor the performance characteristics of the DNAgard[®] Blood Tube as part of an *in vitro* diagnostic procedure have been established. This product's suggested use is blood collection for molecular testing methods that require intact genomic DNA from whole blood. These test methods include, but are not limited to, PCR, SNP genotyping, and DNA sequencing.

Product Summary

Sterile
6.5 mL Draw Volume
1.0 mL DNA stabilization reagent

16x100 mm Plastic Tube

50 Tubes/box (Cat. No: 53561-038)
100 Tubes/box (Cat. No: 53562-048)

Summary and Explanation

Clinical research studies often require blood sample collection at multiple geographic sites under a wide range of conditions. DNAgard[®] Blood Tubes are designed for the immediate stabilization of DNA in blood, providing an efficient method for standardized blood collection, transport and storage of whole blood specimens and isolation of their genomic DNA. This product is optimized for compatibility with silica-membrane-based DNA isolation technologies. High yields of pure DNA are obtained that perform well in a wide range of downstream research applications.

Principle of Procedure

DNAGard[®] Blood Tubes are used for the collection, preservation, storage and shipping of whole blood in a sterile plastic tube. The tube contains 1.0mL of a DNA stabilization reagent. Each tube requires a maximum of 6.5mL of whole blood-draw for a total of 7.5mL mixture. Genomic DNA can be isolated from the stored and preserved blood using commercially available purification kits.

Safety and Warnings

Practice safe laboratory procedures as mandated by your lab. Wear gloves, lab coat and protective eyewear when handling this product. Avoid skin contact with all reagents. In case of contact, wash thoroughly with water. Refer to SDS in case of accidental ingestion or skin contact. All SDS information is available at www.biomatrica.com.

Contents of this tube may cause irritation to eyes, respiratory system and skin.

1. If accidental inhalation occurs, supply fresh air, and seek medical advice in case of complaints.
2. In case of skin contact, immediately wash with water and soap and rinse thoroughly.
3. In case of eye contact, rinse immediately with plenty of water for at least 15 minutes and seek medical advice.
4. If accidental swallowing occurs, immediately seek medical advice.

Specimen Collection and Preparation for Analysis

A. Required Blood Collection Accessories. (Not included with DNAGard[®] Blood Tube)

1. Needle or blood collection set
2. Standard size needle holder for use with 16mm diameter tubes
3. Labels for positive donor identification of samples.
4. Alcohol swab for cleansing site.
5. Dry sterile gauze.

6. Tourniquet.
7. Needle disposal container for used needle or needle/holder combination.

B. Procedure for Specimen Collection

1. Keep the DNAgard[®] Blood Tubes at room temperature (18-25°C) for at least 2 hours prior to blood collection.
2. The DNAgard[®] Blood Tube should be the last tube drawn.
3. Collect blood into the DNAgard[®] Blood Tube using your institution's recommended procedure for standard venipuncture technique (See Prevention of Backflow Section).

Please visit www.biomatrica.com/dnagardblood-tube.php for further instructions.

4. **Ensure that the blood has stopped flowing into the tube before removing the tube from the holder.**
5. After blood collection, gently invert the DNAgard[®] Blood Tube ten (10) times.

See section G for important notes on DNAgard[®] Blood Tube storage after blood collection.

6. DNA purification: Genomic DNA from blood stored in DNAgard[®] Blood performs best when isolated using silica-membrane-based spin column DNA purification kits. Please refer to Appendix A and Appendix B for examples of magnetic bead or salting-out precipitation methods for DNA isolation.

See section below on “DNA recovery from DNAgard[®] Blood Tube” for important notes on processing DNAgard[®] Blood Tubes for DNA isolation.

C. Limitations

1. The use of this tube is restricted to Research Use Only, and therefore the use of this product for diagnostic procedures and patient management is strictly prohibited. Neither the clinical utility nor the performance characteristics of DNAgard[®] Blood Tubes as part of an *in vitro* diagnostic procedure has been established.
2. The quantity of blood drawn varies with altitude, ambient temperature, barometric pressure, tube age, venous pressure, and filling technique.
3. Blood stored in DNAgard[®] Blood is best processed with silica-membrane-based spin column DNA purification kits.

D. Precautions

1. Practice Universal Precautions. Use gloves, gowns, eye protection, other personal protective equipment, and engineering controls to protect from blood splatter, blood leakage, and potential exposure to blood borne pathogens.
2. Handle all biologic samples and blood collection "sharps" (lancets, needles, luer adapters, and blood collection sets) according to the policies and procedures of your facility. Obtain appropriate medical attention in the event of any exposure to biologic samples (for example, through a puncture injury), since they may transmit viral hepatitis, HIV (AIDS), or other infectious diseases. Utilize any built-in used needle protector, if the blood collection device provides one. Biomatrix does not recommend re-shielding used needles. However, the policies and procedures of your facility may differ and must always be followed.
3. Discard all blood collection tubes in biohazard containers approved for their disposal.
4. Do not re-use DNAgard[®] Blood Tubes.
5. Do not use DNAgard[®] Blood Tubes after expiration date printed on the tube label.

6. Since DNAgard[®] Blood Tubes contain a chemical additive, precautions should be taken to prevent possible backflow from the tube during blood drawing (See "Prevention of Backflow" section).
7. DNAgard[®] Blood Tube should be the last tube drawn in the phlebotomy process.
8. Excessive centrifugation speed may cause DNAgard[®] Blood Tube breakage, exposure to blood and possible injury.
9. Transferring a sample from a syringe to a tube is not recommended. Additional manipulation of sharps increases the potential for needlestick injury. In addition, depressing the syringe plunger during transfer can create a positive pressure, forcefully displacing the stopper and sample and causing a potential blood exposure. Using a syringe for blood transfer may also cause over or under filling of tubes, resulting in an incorrect blood-to-additive ratio and potentially incorrect analytic results. The laboratory should be consulted regarding the use of these samples.
10. The use of DNAgard[®] Blood Tubes is contraindicated with an intravenous (I.V.) line. This is critical to avoid erroneous laboratory data from I.V. fluid contamination.

E. Prevention of Backflow

Since DNAgard[®] Blood Tubes contain a chemical additive, it is important to avoid possible backflow from the tube, with the possibility of adverse donor reactions. To guard against backflow, observe the following precautions:

1. Place donor's arm in a downward position.
2. Hold tube with the stopper uppermost.
3. Release tourniquet as soon as blood starts to flow into tube.
4. Make sure tube additives do not touch stopper or end of the needle during venipuncture.

F. Storage of DNAgard[®] Blood Tubes (Prior to blood collection):

Store DNAgard Blood[®] Tubes between +4°C and 25°C until the end of shelf life marked by the expiration dates. Equilibrate the DNAgard[®] Blood Tubes to room temperature (18-25°C) for at least 2 hours prior to blood collection. Do not use tubes after their expiration date. We recommend avoiding exposure to sunlight.

G. Storage of DNAgard[®] Blood Tubes (Post-blood collection):

Genomic DNA is protected by DNAgard[®] Blood stabilization formulation for at least 14 months at room temperature (18 - 25°C) and for extended time periods at 2-8°C or -20°C. However, the duration which blood can be stored in the DNAgard Blood Tube depends on sample processing needs (see Table 1):

Maximum Storage Times in DNAgard[®] Blood Tubes

Storage Temperature	Aliquot processing	Full-tube processing
-20°C	Indefinite	Indefinite
2-8°C	14 months	14 months
18 - 25°C	1 month	14 months

Table 1. The recommended blood storage time based on method of blood sample processing (blood aliquot processing from tube versus full-tube processing). Full tube processing constitutes the entire contents of the blood tube in a single purification. Aliquot processing constitutes using several aliquots in separate purifications.

If freezer storage is desired, follow standard freezer protocols for blood collection tubes. Avoid Styrofoam[®] racks as this may induce cracking of blood collection tubes.

For storage at temperatures below -20°C, use cryo vials.

It is recommended that the cap not be removed from the DNAgard[®] Blood Tube during storage. If the tube is opened, then it is critical that

it be tightly re-sealed and -that the entire cap be wrapped in parafilm to minimize air-flow into the tube.

Special notes for heated samples:

Genomic DNA is protected in blood stored with DNAgard[®] Blood stabilizer even during extreme temperature exposure during shipment (up to 45°C). Precipitation of the blood-stabilizer is expected, but this does not compromise genomic DNA integrity. Because of the challenge precipitation poses to pipetting, if samples are exposed to temperatures above 35°C during shipment, it is recommended to make any desired blood aliquots immediately upon sample arrival in the lab.

DNA recovery from DNAgard[®] Blood Tubes

We recommend silica-membrane-based spin column DNA purification methods for purifying DNA from DNAgard[®] Blood Tubes. Alternate methods are shown in Appendix A and Appendix B.

Vortex DNAgard[®] Blood Tube samples prior to commencing the purification procedure in order to obtain a homogenous re-suspension of any precipitates in the blood-formulation mixture.

We have confirmed compatibility with the following commercially available DNA purification kits:

Large volume processing (up to 7.5 mL)

- QIAamp[®] DNA Blood Maxi Kit (QIAGEN)
- NucleoSpin[®] Blood Kit (Macherey-Nagel)

Small volume processing(≤ 0.2 mL)

- QIAamp[®] DNA Blood Mini Kit (QIAGEN)
- ReliaPrep[™] Blood gDNA Miniprep System (Promega)

When calculating reagent volumes in the purification procedure, the blood-stabilizer mixture should be treated as a whole blood sample.

Appendix A – DNA purification using the FlexiGene[®] DNA Kit

DNA can be recovered from blood stored in DNAgard[®] Blood Tubes using the FlexiGene DNA salting-out procedure with modifications.

FlexiGene reagent volumes used for DNA purification from DNAgard[®] Blood Tube samples differ from the volumes indicated in the FlexiGene DNA handbook (**Table 1**). The pellet size obtained after sample centrifugation in Buffer FG1 is usually larger than typically observed in whole blood. The volumes of Buffer FG2 / Protease cocktail and isopropanol should be doubled relative to the standard FlexiGene Protocol (**Table 1**).

Important points for using the FlexiGene DNA Kit for DNAgard[®] Blood samples:

- Vortex or pipette up and down to thoroughly mix blood samples after the addition of Buffer FG1 and Buffer FG2/ QIAGEN Protease.
- Pipette off the supernatant after sample centrifugation in Buffer FG1 (instead of decanting).
- Double the volume of Buffer FG2/ QIAGEN Protease (**Table 1**).
- Double the heating time at 65°C from 5 minutes to 10 minutes. (The color of the DNAgard[®] Blood samples after heating may be green to dark brown).
- Double the volume of Isopropanol (100%) (**Table 1**).

Scale the reagent volumes listed in the chart below according to the desired starting blood volume. This modified protocol has been tested on small volume DNAgard[®] Blood samples (100 µl – 300 µl).

	Blood Volume (μl)			
	100		200	
	Standard protocol	DNAgard Blood	Standard protocol	DNAgard Blood
Buffer FG1 (μl)	250	250	500	500
Buffer FG2/ QIAGEN Protease (μl)	50	100	100	200
100% isopropanol (μl)	50	100	100	200
70% ethanol (μl)	50	50	100	100
Buffer FG3 (μl)	100	100	200	200

Table 1. FlexiGene DNA Kit reagent volumes for the standard FlexiGene protocol and for the modified protocol recommended for DNAgard[®] Blood samples.

Reagent volumes are shown for two initial blood volumes (100 μl and 200 μl). The blood-DNAgard[®] Blood formulation mixtures should be considered as whole blood for the purpose of calculating reagent volumes.

Appendix B – DNA purification using Roche MagNA Pure Compact automation with the MagNA Pure Compact Nucleic Acid Isolation Kit I

Ensure that sufficient volume of the sample is used for the purification. The higher viscosity in the DNAgard[®] Blood samples might be detected by the MagNA Pure Compact instrument and can result in a ‘fail’ indication at the end of the purification procedure, even though highly pure genomic DNA is recovered. The purified DNA is free of hemin contamination and fully compatible with PCR-based amplification procedures.

Frequently Asked Questions

Situation	Comment	Suggestion
The blood looks coagulated or precipitated after storage or shipment.	DNAgard [®] Blood stabilization formulation denatures proteins. The amount of precipitation is increased by time and elevated temperatures. Precipitation does not reduce the DNA stabilization properties of the formulation.	Cold-storage (4°C or -20°C) reduces the rate of precipitation in the blood sample. We recommend using silica-membrane column-based DNA purification kits.
Can I store or ship my DNAgard blood tubes at 2-8°C, on ice, -20°C, and -80°C?	DNAgard [®] Blood Tubes can be maintained at 2-8°C and -20°C. Storage at -80°C is not recommended as this can cause cracking of the blood tube.	
Can I prepare plasma or serum from DNAgard [®] Blood Tubes?	No. DNAgard [®] Blood Tubes are for the preservation of genomic DNA in whole blood samples.	
Does this product stabilize RNA in blood samples?	No. DNAgard [®] Blood is not optimized for RNA stabilization.	
Low yield of genomic DNA	Possible reasons: - Low concentration of leukocytes in the blood sample - DNAgard [®] Blood Tube sample not homogenized thoroughly prior to aliquoting blood or DNA purification - Choice of DNA isolation method - Automated pipetting of DNAgard [®] Blood samples	Leukocyte concentrations can vary 10-fold between donors. Try isolation from a larger volume of blood. Vortex the DNAgard [®] Blood Tube immediately prior to aliquoting blood or prior to whole-tube purification DNAgard [®] Blood is optimized for purification using silica-membrane column-based kits. If pipetting from DNAgard [®] Blood Tubes is automated, we recommend cold shipment and storage of DNAgard [®] Blood Tubes to minimize precipitation that can cause inaccuracies in sample pipetting.
Isolated DNA is impure	Possible reasons: - Choice of DNA isolation method	DNAgard [®] Blood is optimized for purification using silica-membrane column-based kits. Refer to Appendices for suggestions on using the FlexiGene DNA salting-out protocol or the MagNA Pure Compact for DNA isolation.

<p>Can I purify DNA from DNAGard[®] Blood Tubes using a non-column based method?</p>	<p>Yes although DNAGard[®] Blood is optimized for silica-membrane column-based purification methods.</p> <p>DNA yield and/ or purity can be reduced by the use of other types of purification methods.</p>	<p>Refer to Appendix A for suggestions on using the FlexiGene DNA salting-out protocol for DNA isolation.</p> <p>Refer to Appendix B for suggestions on using the Roche MagNA Pure Compact automated system for DNA isolation</p>
<p>Has DNAGard[®] Blood been tested on automated DNA purification systems?</p>	<p>DNA isolation from DNAGard[®] Blood samples has been tested on the following automated systems:</p> <ul style="list-style-type: none"> - QIAcube with the QIAamp Blood Mini Kit. (QIAGEN) - MagNA Pure Compact with the MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche) 	<p>If automated DNA purification systems are to be used we recommend cold shipment and storage of DNAGard[®] Blood Tubes to minimize precipitation that can affect pipetting accuracy.</p> <p>Vortex blood samples to homogenization immediately prior to starting purification protocol.</p> <p>Refer to Appendix B for suggestions on using the Roche MagNA Pure Compact automated system for DNA isolation</p>

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 products. Please contact Biomatrica directly with any questions regarding DNAGard® technology, product use, or general matters.

Technical Service Department

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

Web: www.biomatrica.com

Email: techsupport@biomatrica.com

Label Information

	Item number		Sterilization Using Irradiation
	LOT number: Batch number		Do Not Reuse
	Expiry Date. Use by the end of the month indicated		Manufacturer

DNAGard® is a registered trademark of Biomatrica.

QIAGEN® and QIAamp® are registered trademarks of the QIAGEN.

ReliaPrep™ is a trademark of Promega.

NucleoSpin® is a registered trademark of Macherey-Nagel GmbH & Co.KG.



Manufactured for: Biomatrica Inc.

5627 Oberlin Drive, #120

San Diego, CA 92121, U.S.A.

by Greiner Bio-One (Kremsmuenster, Austria).

©2016 Biomatrica Inc.

Biomatrica®