

RNAgard[®] Blood Reagent Handbook

For room temperature shipping and storage of RNA in
whole blood

Biomātrica[®]
THE BIOSTABILITY COMPANY

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Product Format

Catalog number	Volume (mL)	Description
66201-151	RNAgard Blood Reagent, 100 mL	100 mL screw cap bottle

Storage

RNAgard Blood Reagent bottles must be stored between 4°C - 25°C.

Quality Control

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

Product Use Limitations

RNAgard Blood Reagent is intended for Research Use Only. Not for use in diagnostic procedures.

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 contact Biomatrix Technical Services or your local distributor (visit www.biomatrix.com).

Safety Information

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 Biomatrix.

Technical Assistance

At Biomatrix, we pride ourselves on the quality and availability of our technical support. Our Technical Service Department is 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 RNAgard Blood Reagent, 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

Phone: USA (866) DRY-MTRX or (866) 379-6879
Email: contact@biomatrix.com
Web: www.biomatrix.com

Introduction

Biological or medical studies often require blood sample collection from human or animals at multiple geographic sites under a wide range of conditions. RNAgard[®] Blood Reagent is designed for the immediate stabilization of cellular RNA in blood. It provides a safe and efficient method for shipping and storage of RNA in whole blood. The reagent provides users with flexibilities for shipping and storage of either human or animal blood samples at room temperature. The reagent effectively preserves cellular RNA from whole blood with unaltered gene expression profiles. Blood RNA isolation kits such as BioMaxi[®] Blood RNA Purification Kit (Cat.# 64201-601, Biomatrix) and RiboPure[™] Blood Kit (Cat.# AM1928, Life Technologies) are recommended for RNA purification from blood stabilized in RNAgard Blood Reagent. The purified RNA can be further applied in a wide range of downstream applications, including but not limited to, bioanalyzer, RT-qPCR and gene expression arrays.

Procedure

1. Add whole blood to a tube pre-aliquoted with RNAgard Blood Reagent making a final ratio of 1:2.5 (blood volume : reagent volume). The final volume of the blood-reagent mixture should not exceed 2/3 of the fill capacity of the tube.

Notes: Immediately close the cap of the reagent bottle when not in use. Tubes that can be used in this procedure include: VACUETTE[®] blood collection tubes, microfuge tubes, and larger tubes (e.g. Falcon[™] tubes).

2. Mix well by inversion at least 5 times. Keep the tube at room temperature for at least 2 hours before storing at lower temperatures. The table below shows maximum storage times for the blood-reagent mixture.

Maximum storage times for blood samples in RNAgard Blood Reagent^{*§}

Storage Temperature	Length of Time
-20°C or -80°C	Long Term
4°C	1 month
18°C - 25°C	14 days

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

§ Cellular RNA is protected in blood stored with RNAgard Blood Reagent even when samples are exposed to high temperatures during shipment (up to 37°C for up to 12 hours).

3. RNA Purification. After blood is collected in RNAgard Blood Reagent, tubes containing the blood-reagent mixture need to be kept at room temperature for at least 2 hours before RNA purification. If tubes containing the blood-reagent mixture are stored at or below 4°C, they must be equilibrated to room temperature before RNA purification. The following kits are recommended for purification of RNA stabilized with RNAgard Blood Reagent:

- BioMaxi[®] Blood RNA Purification Kit (Cat. # 64201-601, Biomatrix) has been optimized for 9.15mL of blood-reagent mixture (2.5mL blood + 6.65mL reagent). Under this condition, the BioMaxi kit provides the highest yields of RNA via a convenient spin column method. A detailed purification protocol is described in the handbook of the RNAgard Blood System (Cat. No 45201-000, Biomatrix).
- RiboPure[™] Blood Kit (Cat. # AM1928, Life Technologies) has been validated for purification of RNA stabilized in RNAgard Blood Reagent. A detailed purification protocol is described in the Appendix of this handbook.

Appendix: Purification of RNA Stabilized in RNAgard Blood Reagent Using RiboPure Blood Kit

Important Notes Before Starting:

- Allow at least 2 hours after blood collection to start the purification process. In order to maximize RNA yield, allow 8-12 hours of storage at room temperature prior to RNA purification.
 - Make sure tubes containing blood-reagent mixtures are equilibrated to room temperature before starting the RNA purification process.
1. Invert tube 3-5 times prior to commencing the extraction procedure to obtain a homogenous re-suspension of any precipitates in the blood-reagent mixture.
 2. Transfer blood-reagent mixture from the tube to a 2mL microfuge tube.
 3. Add 1/3 of the sample volume of RNase-free water and vortex vigorously for 15 seconds.
 4. Centrifuge 5 minutes at 12,000-14,000 rpm and carefully decant the supernatant without disturbing the pellet.
 5. Add 800 μ L of RiboPure Blood Lysis Solution and 50 μ L of RiboPure Blood Sodium Acetate Solution to the pellet, and vortex vigorously for 15 seconds.
 6. Transfer the sample to a 2mL microfuge tube and proceed to the extraction with Acid-Phenol:Chloroform, following the RiboPure Blood Kit protocol from "Section B, Cell Lysis and Initial RNA Purification, Step 3". Follow according to the manufacturer's instructions.

Note: If the pellet is larger than 1mL in Step 5 above, divide the sample into two 2mL microfuge tubes, adjust the volume to 1mL with RiboPure Blood Lysis Buffer and add 62.5 μ L of RiboPure Blood Sodium Acetate Solution to the pellet. Vortex briefly and proceed with Acid-Phenol:Chloroform extractions. Samples can then be combined and processed through the same purification column after addition of the adjusted volume of 100% ethanol.

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VACUETTE[®] is a registered trademark of Greiner Bio-One.
RiboPure[™] is a trademark of Life Technologies, Inc.
All other trademarks cited herein are the property of their respective owners.

Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the RNAgard[®] Blood Reagent to the Terms and Conditions of Sales stated at Biomatrix's website (http://biomatrix.com/terms_and_conditions.php).

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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 DNAstable technology, product use, or general matters.

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