RNAgard® Blood System Handbook

For the collection, preservation and purification of RNA from whole blood at room temperature

For Research Use Only. Not for use in diagnostic procedures.

Biomātrica®
THE BIOSTABILITY COMPANY

RNAgard® Blood System

RNAgard® Blood Tubes

BioMaxi™ Blood RNA Purification Kit

For the collection, preservation and purification of RNA from whole blood at room temperature

Instructions For Use

Protocol – blood collection, storage and processing for cellular RNA isolation

Cat. No: 62201-131 (RNAgard Blood Tubes: 50 tubes)
Cat. No: 62201-141 (RNAgard Blood Tubes: 1200 tubes)
Cat. No: 64201-601 (BioMaxi Blood RNA Purification Kit: 50 preps)
Cat. No: 45201-000 (RNAgard Blood System: 50 tubes + 50 preps)

Contents

Product Summary

The RNAgard Blood System for collection, preservation and purification of RNA from whole blood is composed of RNAgard Blood Tubes and the BioMaxi Blood RNA Purification Kit. RNAgard Blood Tubes are plastic, sterile, evacuated tubes, pre-filled with a stabilization reagent to preserve cellular RNA in whole blood prior to isolation. Purification of RNA from blood stabilized in RNAgard Blood Tubes is optimized with the BioMaxi Blood RNA Purification Kit. This product is restricted to Research Use Only, and is not intended for diagnostic procedures and patient management. Neither the clinical utility nor the performance characteristics of RNAgard Blood Tubes as part of an *in vitro* diagnostic procedure have been established. Its suggested use is for research as well as molecular testing methods that require high quality cellular RNA from whole blood with preserved gene expression.

Kit Contents and Storage

BioMaxi Blood RNA Purification Kit Contents

BioMaxi Blood RNA Purification Kit (50 preps) Catalog No. 64201-601	
Components	**Amount**
RP (Precipitation Buffer)	180 mL
RS (Resuspension Buffer)‡	21 mL
RW 1 (Wash Buffer 1)*	48 mL
RW 2 (Wash Buffer 2)*	44 mL
RNase-Free Water (bottle)	12 mL
DNase I, RNase-Free; Lyophilized Powder	1 X vial
DNase I Buffer	2 X 2.5 mL
Sterile Water (DNase I Resuspension Water)	1 X 1 mL
Purification Columns	50
2ml Collection Tubes	2 X 100

‡ Not compatible with disinfecting reagents containing bleach. Contains guanidine thiocyanate. See page 5 for safety information.

* Buffer RW 1 and RW 2 are supplied as a concentrate. Before using for the first time, add ethanol (96-100%, purity grade p.a.) volumes as indicated on the bottles.

BioMaxi Blood RNA Purification Kit Storage

DNase I, RNase-Free is shipped at ambient temperature as a lyophilized powder. Upon arrival, store at 4°C. Store all other components at ambient room temperature (18°C to 25°C).

RNAgard Blood Tube Product Summary

RNAgard Blood Tube, 2.5 mL draw volume
6.65 mL RNA stabilization reagent, 16x100 mm plastic tube

50 tubes/box (Cat. No: 62201-131)
1200 tubes/box (Cat. No: 62201-141)

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 MSDS in case of accidental ingestion or skin contact. All MSDS 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.

Introduction

Principle of Procedure

RNAgard Blood Tubes are used for the collection, preservation, storage and shipping of whole blood. The tube contains 6.65mL of a RNA stabilization reagent. Each tube requires a maximum of 2.5mL of whole blood-draw for a total mixture volume of 9.15 mL. High quality cellular RNA can be isolated from preserved blood using Biomatrica's BioMaxi Blood RNA Purification Kit.

Summary and Explanation

Clinical Research studies often require blood sample collection at multiple geographic sites under a wide range of conditions. RNAgard Blood Tubes are designed for the immediate stabilization of cellular RNA in human blood samples, providing an efficient method for standardized collection, transport and storage of whole blood specimens and isolation of their RNA material. Purification of RNA from blood stabilized in RNAgard Blood Tubes has been optimized with the BioMaxi Blood RNA Purification Kit, and is not recommended for use with other RNA purification methods. High yields of high quality RNA with unaltered gene expression are obtained, and perform well in a wide range of downstream research applications, including but not limited to, bioanalyzer, gene quantification by qPCR and gene expression arrays.

Specimen Collection and Preparation for Analysis

A. Required Blood Collection Accessories. (Not included with RNAgard Blood System)

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 injection 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 RNAgard Blood Tubes at room temperature (15°C to 25°C) for at least 2 hours prior to blood collection.
2. RNAgard Blood Tubes should be the last tubes drawn.
3. Collect blood into RNAgard Blood Tubes using your institution's recommended procedure for standard venipuncture technique (See Prevention of Backflow Section).

   Please visit www.biomatrica.com/ifu.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 RNAgard Blood Tubes five times.

   See section G for important notes on RNAgard Blood Tube storage after blood collection.
6. RNA purification: Cellular RNA from blood stored in RNAgard Blood Tubes should be purified using Biomatrica's BioMaxi Blood RNA Purification Kit.

   See section below on "RNA recovery from RNAgard Blood Tubes" for important notes on processing RNAgard Blood Tubes for RNA isolation.

C. Limitations

1. The use of this product is restricted to Research Use Only, and therefore its use for diagnostic procedures and patient management is strictly prohibited. Neither the clinical utility nor the performance characteristics of RNAgard Blood Tubes as part of an *in vitro* diagnostic procedure have been established.
2. The quantity of blood drawn varies with altitude, ambient temperature, barometric pressure, tube age, venous pressure, and filling technique.

3. For maximum RNA yield and quality, blood stored in RNAgard Blood Tubes is recommended to be processed using Biomatrica's BioMaxi Blood RNA Purification kit.

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. Biomatrica 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 RNAgard Blood Tubes.
5. Do not use RNAgard Blood Tubes after the expiration date printed on the tube label.
6. Since RNAgard 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. RNAgard Blood Tubes should be the last tubes drawn in the phlebotomy process.
8. Excessive centrifugation speed may cause RNAgard 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 RNAgard 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 RNAgard 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 RNAgard Blood Tubes (Prior to blood collection):

Store RNAgard Blood Tubes between 4°C and 25°C until the end of shelf life marked by the expiration dates. Equilibrate the RNAgard 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 RNAgard Blood Tubes (Post-blood collection):

RNA is protected by RNAgard Blood stabilization formulation for at least 14 days at room temperature (18-25°C), 1 month at 4°C and for extended time periods at -20°C or -80°C.

Maximum Storage Times in RNAgard Blood Tubes

Storage Temperature	Full Tube Processing Time
-20°C or -80°C	Long Term
4°C	1 month
18°C - 25°C	14 days

Table 1. Recommended blood storage time according to storage temperature of stabilized blood sample

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

It is recommended that the cap not be removed from the RNAgard Blood Tube during storage. If the tube is opened, it is critical that it be tightly re-sealed and wrapped in parafilm to minimize air-flow into the tube.

Special notes for heated samples:
Cellular RNA is protected in blood stored with RNAgard Blood stabilizer even when samples are exposed to high temperatures during shipment (up to 37°C for up to 12h).

H. Storage of BioMaxi Blood RNA Purification Kit:

DNase I, RNase-Free, is shipped at ambient temperature as a lyophilized powder. Upon receiving the BioMaxi Blood RNA Purification Kit, remove DNase I and store at 4°C. The lyophilized powder will be resuspended to make the DNAse I stock solution. After resuspension, store at -20°C (DNase I is sensitive to physical denaturation; do not vortex the resuspended DNase I). Store all other components of BioMaxi Blood RNA Purification Kit at room temperature.

RNA purification from RNAgard Blood Tubes using BioMaxi Blood RNA Purification Kit

Important Notes Before Starting:

- Ensure that blood was collected in RNAgard Blood Tubes according to the instructions in the “Specimen Collection and Preparation for Analysis” section.
- 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 ambient temperature prior to RNA purification.
- Wash Buffer 1 and Wash Buffer 2 are provided as concentrates; reconstitute with the indicated amounts of 100% ethanol prior to use.
- Make sure the RNAgard Blood Tubes are equilibrated to room temperature before starting the RNA purification process.

If using DNase I for the first time, prepare DNase I stock solution by adding 300 µL of sterile, RNase-free Water (provided) to the DNase I (RNase-Free) lyophilized powder and mix gently. Aliquot the DNase I enzyme and store at -20°C for long term storage. **Note: The DNase I stock can be freeze/thawed up to three times without loss of activity.**

1. Invert the RNAgard Blood Tube 3-5 times to ensure proper mixing, remove the cap and pour the contents of the tube into a clean 50 mL conical tube.

2. Pipet 3 mL of Precipitation Buffer into the 50 mL conical tube to bring the total volume to ~12 mL and close the cap on the tube.

3. Incubate 15 minutes at room temperature with shaking (500-750 rpm).

4. Vortex the 50 ml conicaL tube vigorously (maximum speed) for at least 30 seconds, ensuring that the solution travels to the top of the tube to achieve proper mixing of the contents.

5. Centrifuge the tube at >4500g for 30 minutes at room temperature in a swing-bucket rotor.

   Note**:** *Similar RNA yields can be obtained by centrifugation at 9000g for 10 minutes at room temperature, using a fixed-angle rotor. Centrifugation can also be* performed at 4°C, without *impact on RNA yield or quality.*

6. Carefully pour off the supernatant and leave the tube inverted on absorbent paper for 1-2 minutes.

   Note: *A translucent reddish pellet should be visible at the bottom of the tube.*

7. Blot the remaining drops of liquid off the rim of the tube with clean absorbent paper.

8. Add 350 µL of Resuspension Buffer into the tube and pulse-vortex 5-10 times to resuspend the pellet.

9. Add 250 µL of 100% ethanol into the tube and pulse-vortex 3-5 times to mix the contents of the tube.

10. Transfer solution to a clean purification column, close the lid and centrifuge for 1 minute at 8000 rpm.
    Note*: For any centrifugation (between steps 9 – 23) use a bench-top microfuge at room temperature.*

11. Discard the collection tube and place column in a clean 2 mL collection tube.

12. Add 400 µL of Wash Buffer 1 into the column, close the lid and centrifuge at 8000 rpm for 1 minute.

13. Discard the flow-through, place column into the same 2 mL collection tube and centrifuge at 14000 rpm for 1 minute to dry the column.

14. Discard the collection tube and place column in a clean 2 mL collection tube.

15. For each RNA sample, mix 5 µL of DNase I and 95 µL of DNase I Buffer to prepare DNase I Mix.

***Example**: For 10 samples, mix 50 µL of DNase I and 950 µL of DNase I Buffer.*

16. Add 100 µL of the DNase I Mix into the column and incubate at room temperature for 15-20 minutes.

    ***Note**: Preheat the RNase-free water for elution to 70°C during this step.*

17. Add 400 µL of Wash Buffer 1 to the column, close the lid, incubate at room temperature for 30 seconds and centrifuge at 8000 rpm for 1 minute.

18. Discard the collection tube and place column in a clean collection tube.

19. Add 350 µL of Wash Buffer 2 to the column, close the lid and centrifuge at 8000 rpm for 1 minute.

20. Repeat step 18 with a second 350 µL of Wash Buffer 2**,** without changing collection tube.

21. Discard the collection tube, place column in a clean 2 mL collection tube and centrifuge at 14000 rpm for 2 minutes to dry the column.

22. Remove the purification column from the tube and place in an RNase-free 1.5 mL microfuge tube (not provided in the kit) for eluting the RNA.

23. Add 100 µL of RNase-free water onto the column, close the lid, and incubate for 1 minute at room temperature and centrifuge for 1 minute at 8000 rpm to elute RNA.

24. For maximum RNA concentration, re-apply the eluted solution back to the column and centrifuge 1 minute at 8000 rpm. For maximum RNA yield, repeat the step 22 with new 100 µL of RNase-free water.

Frequently Asked Questions

Situation	Comment	Suggestion
The blood looks coagulated or precipitated after storage or shipment.	RNAgard Blood formulation denatures proteins, which will precipitate over time. Precipitation does not reduce the RNA stabilization properties of the formulation.	Ensure that sample is mixed before RNA purification procedure, by inverting tube 3-5 times.
Can I store or ship my RNAgard Blood Tubes at 4°C, on ice, -20°C, and -80°C?	RNAgard Blood Tubes can be maintained at 4°C, on ice and -20°C. However, -80°C storage can cause cracking of the blood tube.	For storage at temperatures below -20°C, use cryo vials, in order to avoid cracking of the blood tubes.
Can I prepare plasma or serum from RNAgard Blood Tubes?	No. RNAgard Blood Tubes are for the preservation of RNA in whole blood samples. Blood cells will be lysed and therefore plasma cannot be prepared.	
Low yield of RNA	Possible reasons: - Low concentration of leukocytes in the blood sample - RNAgard Blood Tube sample not homogenized thoroughly prior to aliquoting blood or extraction - Choice of RNA isolation method	Leukocyte concentrations can vary 10-fold between donors. Invert the RNAgard Blood Tube 3-5 times immediately prior to RNA isolation. RNA isolation from RNAgard Blood Tubes has been optimized with the BioMax Blood RNA Purification Kit. We do not guarantee the quality of RNA isolated with a different kit.
Isolated RNA is impure	Possible reasons: - Choice of RNA isolation method	RNA isolation from RNAgard Blood Tubes has been optimized with the BioMax Blood RNA Purification Kit. We do not guarantee the quality of RNA isolated with a different kit.
Can I purify RNA from RNAgard Blood Tubes using a different isolation method?	We do not guarantee the quality of RNA isolated with a different kit. RNA isolation from RNAgard Blood Tubes has been optimized with the BioMaxi™ Blood RNA Purification Kit.	

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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 RNAgard technology, product use, or general matters.

Technical Service Department
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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

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