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Preserved Blood RNA Purification Kit

Product Insert

(for use with Norgen Blood RNA Preservative Tubes)

Product # 52600, 52700

Intended Use

Norgen's **Preserved Blood RNA Purification Kit (for use with Norgen Blood RNA Preservative Tubes)** is intended for the isolation and purification of total RNA from blood that has been preserved using Norgen's Blood RNA Preservative Tubes. Please see Norgen's **Blood RNA Preservative Tubes** Product Insert for detailed information about the use of Norgen's Blood RNA Preservative Tubes.

Summary

Norgen has developed a system that enables the collection, stabilization, storage, and transportation of whole blood specimens, together with a rapid and efficient protocol for purification of intracellular RNA. The system requires the use of Norgen's **Blood RNA Preservative Tubes** for blood collection and RNA stabilization, followed by RNA purification using Norgen's **Preserved Blood RNA Purification Kit (for use with Norgen Blood RNA Preservative Tubes).** Collection of whole blood is the first step in many molecular assays used to study cellular RNA and gene expression. However, a major problem in such assays is the instability of the cellular RNA profile *in vitro*. Studies have shown that the copy numbers of individual mRNA species in whole blood can change more than 1000-fold during storage or transport at room temperature, due to rapid RNA degradation and by induced expression of certain genes after the blood is drawn. Such changes in the RNA expression profile interfere with reliable studies of gene expression. A method that preserves the RNA expression profile is therefore essential for accurate analysis of gene expression in human whole blood.

Performance Characteristics of Norgen's Blood RNA Preservative Tubes

After blood has been collected into Norgen's Blood RNA Preservative Tubes, the intracellular RNA profile remains stable for 12 days at room temperature (18°C to 25°C), 21 days at 4°C, and for extended periods of time at -20°C and below.

Isolation of RNA from Preserved Blood Samples

Norgen's Preserved Blood RNA Purification Kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other cellular components such as proteins, without the use of phenol or chloroform. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

Specifications

Kit Specifications	
Maximum Column Binding Capacity	50 μg
Maximum Column Loading Volume	650 μL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Time to Complete 10 Purifications	45 minutes
Average Yield	6 – 25 μg per 3 mL preserved human blood

Kit Components:

Component	Product # 52600 (48 preps)	Product # 52700 (96 preps)
RNA Extraction Buffer A	75 mL	2 x 75 mL
RNA Extraction Buffer B	75 mL	2 x 75 mL
Resuspension Solution B	30 mL	2 x 30 mL
Wash Solution A	38 mL	2 x 38 mL
Elution Solution A	6 mL	2 x 6 mL
Mini Spin Columns	50	100
Collection Tubes	50	100
Elution tubes (1.7 mL)	50	100
Product Insert	1	1

Customer-Supplied Reagents and Equipment

- Norgen's Blood RNA Preservative Tubes (please see Related Products table)
- Centrifuge (Temperature controlled) with a swinging bucket rotor capable of 6,000 RPM
- Benchtop microcentrifuge
- Vortexer
- Micropipettes with an accuracy range between 10-100 µL and 100-1000 µL
- Disposable powder-free gloves
- Sterile, nuclease-free aerosol-barrier micropipettor tips
- 96 – 100% ethanol
- β-mercaptoethanol
- 1x PBS (Ca2+/Mg2+-free)
- 50 mL Conical Tubes

Storage Conditions and Product Stability

The Blood RNA Extraction Buffer A and RNA Extraction Buffer B should be kept tightly sealed and stored upon arrival at 4^{o}C for up to 1 year without showing any reduction in performance. All other kit components should be kept tightly sealed and stored at room temperature (18-25^{o}C) for up to 1 year without showing any reduction in performance.

General Precautions

- Follow universal precautions. Use gloves, eye protection, and other personal protective equipment to protect from blood splatter, blood leakage and possible exposure to bloodborne pathogens.
- Handle all biological samples and blood collection sharps safely and according to the policies and procedures of your facility. Obtain appropriate medical attention in the event of any exposure to biological samples, since they may transmit HIV (AIDS), viral hepatitis, or other infectious disease.
- Dispose of unused kit reagents and human specimens according to local, provincial or federal regulations.
- Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.
- Do not substitute or mix reagents from different kit lots or from other manufacturers.
- Do not interchange reagent tube / bottle caps as this may lead to contamination and compromise test results.
- Only use the protocol provided in this insert. Alterations to the protocol and deviations from the times and temperatures specified may lead to erroneous results

Quality Control
In accordance with Norgen's ISO 9001 and ISO 13485-certified Quality Management System, each lot of Norgen's Preserved Blood RNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations
For research use only. Not for use in diagnostic procedures. The Preserved Blood RNA Purification Kit is intended for purification of intracellular RNA from human whole blood (4.8×10^6 – 1.1×10^7 leukocytes/ml) for gene expression applications. It is not for the purification of genomic DNA or viral nucleic acids from human whole blood.

Product Warranty and Satisfaction Guarantee
NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

Safety Information
Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

To avoid the risk of infection (e.g., from HIV or hepatitis B viruses) or injury when working with biological and chemical materials, always wear a suitable lab coat, disposable gloves, and protective goggles.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

The **Resuspension Solution B** contains guanidine hydrochloride, and should be handled with care. Guanidine hydrochloride forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

The preserved blood can be disinfected using 1 volume of commercial bleach solution (5% sodium hypochlorite) per 9 volumes of the **Blood RNA Preservative** solution and blood mixture.

Sample-preparation waste, such as supernatants from centrifugation steps in the RNA purification procedure, is to be considered potentially infectious. Before disposal, the waste must be autoclaved or incinerated to destroy any infectious material.

Working with RNA
RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- The RNA area should be located away from microbiological work stations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA samples, ensure that they remain on ice during downstream applications

Procedures

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Notes prior to use:

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96-100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of **Resuspension Solution B**. β -mercaptoethanol is toxic and should be dispensed in a fume hood.
- It is important to work quickly during this procedure.

- **IMPORTANT!**
  - If the preserved blood sample was less than 9 mL, make up the difference by adding enough 1x PBS (Ca^{2+}/Mg^{2+}-free) to bring the total volume to 9 mL
  - If the preserved blood sample was more than 9 mL, discard the difference by pipeting enough to bring the total volume to 9 mL
  - Add **RNA Extraction Buffer A** and **RNA Extraction Buffer B** in the same sequence outlined in **Step 1** below

1. Transfer the preserved blood to a 50 mL conical tube by decanting.

2. Add **1.5 mL RNA Extraction Buffer A** followed by the addition of **1.5 mL RNA Extraction Buffer B** to the preserved blood to bring the volume to **12 mL**.

3. Replace the cap on the tube, then **vortex** the tube vigorously (at maximum vortex speed) for at least **30 seconds** to ensure proper mixing.

IMPORTANT! Vortexing for less than 30 seconds may cause clogging of the spin-column
IMPORTANT! The tube **MUST** be held vertically during vortexing (NOT TILTED) to ensure that the lysate travels to the top of the tube. **Tilting the preserved blood mixture during vortexing will cause inadequate mixing that will lead to poor RNA isolation**
Note: Frothing of the sample after vortexing is normal

4. Incubate immediately at **-20°C for 10 minutes.**

5. Centrifuge the tube at **4°C** at 3000 – 5000 x g (minimum 4500 rpm) on a Beckman JB-6 or equivalent swing bucket centrifuge for 30 minutes.

6. Carefully pour off the supernatant.

IMPORTANT! Handle the tubes carefully so that you do not dislodge the RNA pellet from the bottom of the tube.
Note: The RNA pellet is transparent and invisible.

7. Leave the tube inverted on absorbent paper for 1 to 2 minutes.

8. Blot the remaining drops of liquid off the rim of the tube with a clean, absorbent paper

9. Pipette **570 µL** of **Resuspension Solution B** into the tube, then vortex briefly to resuspend the RNA pellet.

IMPORTANT! To prevent washing any blood residue down the inside of the tube, insert the pipette tip into the tube and add the resuspension solution to the bottom of the tube.

10. The resuspended RNA can be kept on ice while preparing for the next steps.

11. Assemble a spin column with one of the provided collection tubes.

12. To the resuspended RNA, add **330 µL 96-100% Ethanol**, then vortex briefly.

13. Pipet **450 µL** of the mixture from **Step 12** into the Mini Spin column then centrifuge for 1 minute at **≥ 3,500 x g (~6,000 RPM).** Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at 14,000 x g (~14,000 RPM).

14. Discard the flowthrough. Reassemble the spin column with its collection tube

15. Repeat **Step 13 and Step 14** to completely transfer the rest of the mixture from **Step 12.**

16. Apply **400 µL of Wash Solution A** and centrifuge for 1 minute

17. Discard the flowthrough and reassemble the spin column with its collection tube.

Optional Step:
Norgen's Preserved Blood RNA Purification Kit isolates total blood RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Column DNA Removal Protocol** is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step. This step should be performed at this point in the protocol.

18. Repeat Step 16 and Step 17 to wash the Mini Spin Columns a second time.

19. Wash Mini Spin Column a third time by adding another **400 µL of Wash Solution A** and centrifuging for 1 minute.

20. Discard the flowthrough and reassemble the Mini Spin Column with its collection tube.

21. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

22. Place the column into a fresh 1.7 mL Elution tube provided with the kit.

23. Add **100 µL of Elution Solution A** to the column. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by **2 minutes at 5,800 x g (~8,000 RPM).** Note the volume eluted from the column. If the entire 100 µL has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

Storage of RNA

- The purified RNA sample may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

Rapid Flow Chart Procedure

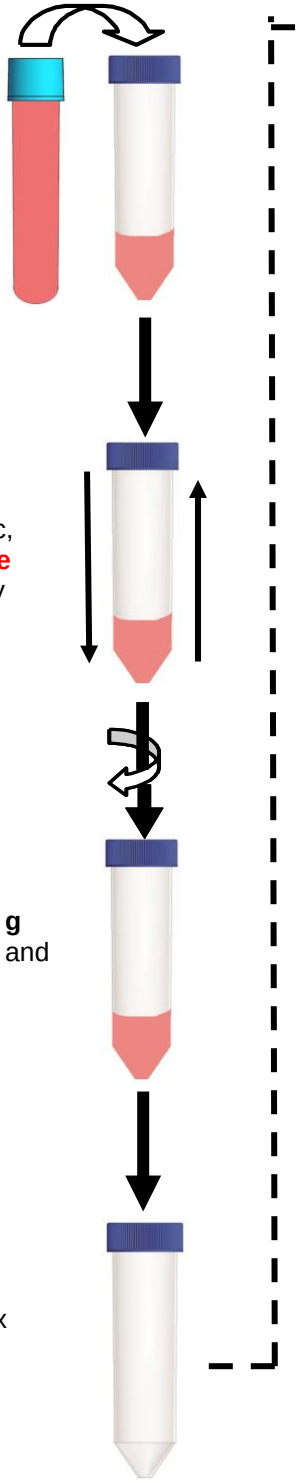
1) Transfer Stabilized Blood to 50 mL Conical Tube then dilute with 1.5 mL **RNA Extraction Buffer A** and 1.5 mL **RNA Extraction Buffer B**

2) Vortex vigorously for at least 30 sec, ensuring that **the lysate travels to the top of the tube**. Incubate immediately at **-20°C for 10 minutes**

3) Centrifuge at **4°C at 3000 – 5000 x g (minimum 4500 rpm)** for 30 minutes, and then carefully pour off supernatant

4) Resuspend RNA pellet in 570 µL **Resuspension Solution B** then vortex briefly

5) Add 330 µL **96-100% Ethanol** then vortex briefly



6) Transfer the resuspended pellet into a Mini Spin Column

Centrifuge at ≥ 3,500 x g (~6,000 RPM), 1 min

7) Wash Three times with 400 µL **Wash Solution A**

Centrifuge at 14,000 x *g* (~14,000 RPM), 1 min

8) Mini Spin Column Drying

Centrifuge at 14,000 x *g* (~14,000 RPM), 2 min

9) Elute Blood RNA with 100 µL **Elution Solution A**

Centrifuge at 200 x g (~2,000 RPM), 2 min
Centrifuge at 5,800 x g (~8,000 RPM) 2 min

Purified Total Blood RNA
Store at –20 °C, or –80 °C for long-term storage

Appendix A

Protocol for Optional On-Column DNA Removal

Norgen's Preserved Blood RNA Purification Kit isolates total blood RNA with minimal amounts of genomic DNA contamination. However, an optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

1. For every on-column reaction to be performed, prepare a mix of 15 µL of DNase I and 100 µL of **Enzyme Incubation Buffer A** using Norgen's RNase-Free DNase I Kit (Product # 25710). Mix gently by inverting the tube a few times. DO NOT VORTEX.

   Note: If using an alternative DNase I, prepare a working stock of 0.25 Kunitz unit/µL RNase-free DNase I solution according to the manufacturer's instructions. A 100 µL aliquot is required for each column to be treated.

2. Perform the **Blood RNA Purification Procedure** up **Step 7** and reassemble the Mini Spin Column with a new collection tube.

3. Apply 100 µL of the RNase-free DNase I solution prepared in **Step 1** to the column and centrifuge at **14, 000 x g (~14 000 RPM) for 1 minute.**

   Note: Ensure that the entire DNase I solution passes through the column. If needed, spin at **14, 000 x g (~14 000 RPM)** for an additional minute.

4. After the centrifugation in **Step 3**, pipette the flowthrough that is present in the collection tube back onto the top of the column.

   Note: Ensure **Step 4** is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

5. Incubate the column assembly at 25 - 30°C for 15 minutes.

6. Without any further centrifugation, proceed directly to the **Step 8** from the Preserved Blood RNA Purification Procedure

Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Preserved Blood RNA Isolation Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable 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 NORGEN. 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 our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. or call one of the NORGEN local distributors (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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