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Preserved Blood RNA Purification Kit I
(for use with Tempus™ Blood RNA Tubes)

Product Insert
Product # 43400

Norgen's Preserved Blood RNA Purification Kit I provides a rapid method for the isolation and purification of total RNA from blood that has been preserved using Tempus™ Blood RNA Tubes*. The 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.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves first lysing the preserved blood using the provided Lysis Solution (please see the flow chart on page 4). Ethanol is then added to the lysate, and the solution is loaded onto a spin-column. Norgen's resin binds RNA in a manner that depends on ionic concentrations. Thus only the RNA will bind to the column, while the contaminating proteins will be removed in the flowthrough or retained on the top of the resin. The bound RNA is then washed with the provided Wash Solution in order to remove any remaining impurities, and the purified total RNA is eluted with the Elution Solution. The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

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	20 minutes
Average Yield	5 – 25 µg per 3 mL preserved human blood

Advantages

- Fast and easy processing of preserved blood using rapid spin-column format
- Isolate total RNA, from large rRNA down to microRNA (miRNA)
- No phenol or chloroform extractions
- Isolate high quality total RNA

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.

**** Tempus™ is a registered trademark of Life Technologies***

Kit Components

Component	Product # 43400 (50 preps)
Tempus™ Blood RNA Tube Diluent	2 x 90 mL
Lysis Solution	40 mL
Wash Solution	22 mL
Elution Solution	6 mL
Mini Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Precautions and Disclaimers
This kit is designed for research purposes only. It is not intended for diagnostic use.

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*.

Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood.

The **Lysis Solution** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Customer-Supplied Reagents and Equipment
You must have the following in order to use the Total RNA Purification Kit:

- Benchtop microcentrifuge
- Swing bucket centrifuge
- 50 mL conical tubes
- 95 - 100% ethanol

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

Flowchart

Procedure for Purifying Total RNA from Preserved Blood using Norgen's Preserved Blood RNA Purification Kit

Pellet cells from preserved blood sample.

Add **Lysis Solution**

Add Ethanol

Bind to column

SPIN

Wash three times with Wash Solution

SPIN

Elute RNA with Elution Solution

SPIN

Purified Total RNA

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

- Refer to manufacturer's product documentation for specific instructions on venipuncture technique, blood collection, storage and shipment conditions and safety information.
- 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** by adding 50 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 72 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- It is important to work quickly during this procedure.
- Blood of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with whole blood.

1. Lysate Preparation from Tempus™ Blood RNA Tubes

1. Lysate Preparation from Tempus™ Blood RNA Tubes

a. Pour the entire contents of the Tempus™ tube into a new 50 mL conical tube.
b. Add 3 mL of **Tempus™ Blood RNA Tube Diluent** (or enough to adjust the final volume to 12 mL). Close the tube tightly and mix by vortexing vigorously for 30 seconds.
c. 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.
d. Discard supernatant. Note that the RNA pellet is transparent and invisible.
e. Leave the tube inverted on paper towel for 2 minutes to dry off excessive liquid.
f. Add 600 µL of **Lysis Solution** to the RNA pellet. Vortex the tube for a few seconds to resuspend the pellet.
g. Add 300 µL of 95-100% ethanol (provided by the user). Vortex briefly to mix.
h. Proceed to "Step 2:Binding RNA to Column".

2. Binding RNA to Column

a. Assemble a column with one of the provided collection tubes
b. Apply up to 600 μL of the lysate with the ethanol (from **Step 1**) onto the column and centrifuge for 1 minute at **≥ 3,500 x g (~6,000 RPM).**

Note: 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).**

c. Discard the flowthrough. Reassemble the spin column with its collection tube.
d. Repeat Step **2b** and **2c** as necessary.

Optional Step:

Norgen's Preserved Blood RNA Purification Kit isolates total 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.

3. Column Wash

a. Apply 400 μL of **Wash Solution** to the column and centrifuge for 1 minute.

Note: Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Repeat steps **3a** and **3b** to wash column a second time.
d. Wash column a third time by adding another 400 μL of **Wash Solution** and centrifuging for 1 minute.
e. Discard the flowthrough and reassemble the spin column with its collection tube.
f. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

4. RNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 μL of **Elution Solution** to the column.
c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by 1 minute at **14,000 x *g* (~14,000 RPM)** Note the volume eluted from the column. If the entire 50 μL has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

Note: For maximum RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 4b** and **4c**).

6. 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.

Appendix A

Protocol for Optional On-Column DNA Removal

Norgen's Preserved Blood RNA Purification Kit isolates total 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** 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 appropriate Preserved Blood RNA Isolation Procedure for your preserved blood sample up to and including "**Binding to Column**" (Steps 1 and 2 of protocol).

3. Apply 400 µL of **Wash Solution** to the column and centrifuge for 2 minute. Discard the flowthrough. Reassemble the spin column with its collection tube.

4. 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.

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

   Note: Ensure Step 5 is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

6. Incubate the column assembly at 25 - 30^{o}C for 15 minutes.

7. Without any further centrifugation, proceed directly to the second wash step in the "**Column Wash**" section (Step 3c).

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of blood	Ensure that the appropriate amount of Lysis Solution was used.
	An alternative elution solution was used	It is recommended that the Elution Solution supplied with this kit be used for maximum RNA recovery.
	Ethanol was not added to the lysate	Ensure that the appropriate amount of ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution	Ensure that 50 mL of 95% ethanol is added to the supplied Wash Solution prior to use.
	Insufficient centrifugation time and speed when pelleting the stabilized blood RNA before column purification	Centrifuge the samples for at least 30 minutes or longer at 4,000 x *g* or higher.
	The RNA pellet was lost during decanting	Pour off the supernatant slowly and carefully.
Clogged Column	Insufficient solubilization of blood	Ensure that the appropriate amount of Lysis Buffer was used.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the columns to clog.
RNA is Degraded	RNase contamination	RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to "*Working with RNA"* at the beginning of this user guide.
	Procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly.
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at –20°C for a few days. It is recommended that samples be stored at –70°C for longer term storage.

Problem	Possible Cause	Solution and Explanation
RNA does not perform well in downstream applications	RNA was not washed 3 times with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed 3 times with Wash Solution. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
Genomic DNA contamination	Large amounts of starting material used	Perform RNAse-free DNaseI digestion on the RNA sample after elution to remove genomic DNA contamination. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

Related Products	Product #
RNase-Free DNase I Kit	25710
Total RNA Purification Kit	17200
RNA/Protein Purification Kit	23000
RNA/DNA/Protein Purification Kit	23500
Cytoplasmic & Nuclear RNA Purification Kit	21000
Leukocyte RNA Purification Kit	21200
microRNA Purification Kit	21300
100b RNA Ladder	15002
1kb RNA Ladder	15003

Technical Support
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.
Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

Norgen's purification technology is patented and/or patent pending. See www.norgenbiotek.com/patents

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