NORGEN BIOTEK CORPORATION

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

Stool Total RNA Purification Kit — Product Insert

Product # 49500

Norgen's Stool Total RNA Purification Kit provides a convenient and rapid method to purify total RNA from small amounts of stool samples. All types of stool samples can be processed with this kit, including animal fecal samples and manure. The kit removes all traces of humic acid using the provided Bead Tubes and a combination of chemical and physical homogenization and lysis. A simple and rapid spin column procedure is then used to further purify the RNA. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA and small interfering RNA. The protocol does not rely on the use of phenol or chloroform, thereby providing a user-friendly procedure and allowing high-throughput analysis on the lab bench. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR and reverse transcription PCR for gene expression analysis.

Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The process involves first adding the stool sample and Lysis Buffer C to a provided Bead Tube, and the tube is vortexed for 5 minutes in order to efficiently and rapidly homogenize the sample and extract the RNA. The sample is then centrifuged, and the supernatant is transferred to a RNase-free microcentrifuge tube. An equal volume of ethanol is 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. An optional on-column DNase treatment can be performed at this point also to remove any residual DNA. The bound RNA is then washed with the provided Wash Solution A in order to remove any remaining impurities, and the purified total RNA is eluted with the Elution Buffer E. The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

Kit Components

Component	Product # 49500 (50 preps)
Lysis Buffer C	60 mL
Wash Solution A	38 mL
Elution Buffer E	6 mL
Bead Tubes	50
Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Advantages

- No phenol or chloroform extractions
- Fast and easy processing (less than 30 minutes for purification)
- Isolate high quality total RNA from a variety of stool samples
- Process all types of feces from humans and animals

Specifications

Kit Specifications	
Maximum Stool Input	200 mg fresh or frozen stool
Type of Stool Processed	All type of feces from humans and animals
Maximum Column Binding Capacity	50 μg
Maximum Column Loading Volume	600 μL
Time to Complete 10 Purifications	30 minutes

Storage Conditions and Product Stability
All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment.

Precautions and Disclaimers
This kit is designed for research purposes only. It is not intended for human or 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*.

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

- Benchtop microcenrifuge
- RNAse-free microcentrifuge tubes
- Flat bed vortex or bead beater equipment
- 96-100% ethanol
- 70% ethanol

Flow Chart

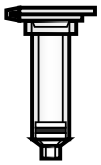
Procedure for Purifying Stool Total RNA using Norgen's Stool Total RNA Purification Kit

Add stool sample and Lysis Buffer C to Bead Tube

↓ Vortex for 5 minutes. Centrifuge. Transfer lysate.

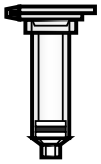
Add Ethanol

↓



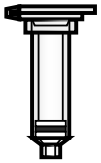
Bind to column

↓ **SPIN**



Wash three times with Wash Solution A

↓ **SPIN**



Elute RNA with Elution Buffer E

↓ **SPIN**

Purified Total RNA

Procedure

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.

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

Notes Prior to Use

- All centrifugation steps are carried out in a benchtop microcentrifuge at **20,000 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 **Wash Solution A** by adding 90 mL of 96 - 100 % ethanol (provided by the user) to each 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.
- The recommended input amount of stool sample is 200 mg.

1. Lysate Preparation

a. Add 200 mg of stool sample to a Bead Tube and add 1 mL of **Lysis Buffer C**. Vortex briefly to mix stool and Lysis Buffer C.
b. Secure tube horizontally on a flat-bed vortex pad with tape, or secure the tube in any commercially available bead beater equipment (e.g. Scientific Industries' Disruptor Genie™). Vortex for 5 minutes at maximum speed or optimize the conditions depending on the machine.
c. Centrifuge the tube for 3 minute at **20,000 × g (~14,000 RPM)**.

d. Transfer up to 600 μL of supernatant to an RNAase-free microcentrifuge tube (not provided).

Note: Avoid any contact with the pellet when collecting the supernatant. Also, depending on the stool type, some reside may be present on top of the supernatant. It is important to avoid collection of this residue while collecting the supernatant.

e. Add an equal volume of 70% ethanol (provided by the user) to the lysate collected above (100 μL of ethanol is added to every 100 μL of lysate). Vortex to mix. **Proceed to Step 2.**

2. Binding to Column

a. Assemble a spin column with one of the provided collection tubes.
b. Apply up to 600 μL of the clarified lysate with ethanol onto the column and centrifuge for 1 minute at **20,000 × g (~14,000 RPM**). Discard the flowthrough and reassemble the spin column with the collection tube.

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.

c. Repeat step **2b** with remained sample in order to bind the entire sample to the column.

Optional Step:

Norgen's Stool Total 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. This step should be performed at this point in the protocol

3. Column Wash

a. Apply 400 μL of **Wash Solution A** 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 **3a** and **3b**.
d. Wash column a third time by adding another 400 μL of **Wash Solution A** 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 75 μL of **Elution Buffer E** to the column.
c. Centrifuge for 2 minutes at **425 x *g* (~2,000 RPM)**, followed by a 1 minute spin at **20,000 x *g* (~14,000 RPM)**. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at **20,000 x g (~14,000 RPM)** for 1 additional minute.

5. Storage of RNA

The purified RNA 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 Stool Total RNA Purification Kit isolates total RNA with minimal amounts of 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 an RNase-free DNase I be used, such as Norgen's RNase-Free DNase I Kit (Product # 25710).

1. Prepare a working stock of 0.25 Kunitz unit/□L RNase-free DNase I solution according to the manufacturer's instructions. A 100 □L aliquot is required for each column to be treated. Alternatively, dissolve or dilute stock DNase I in a reaction buffer (40 mM Tris pH 7.0, 10 mM $MgCl_2$ and 3 mM $CaCl_2$, made RNase-free) to give a final concentration of 0.25 Kunitz unit/□L.
2. Perform the Stool Total RNA purification Procedure up to "**Binding to Column**"
3. Apply 400 µL of **Wash Solution A** to the column and centrifuge for 2 minute. Discard the flowthrough. Reassemble the spin column with its collection tube.
4. Apply 100 □L 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°C for 15 minutes.
7. Without any further centrifugation, proceed directly to the "**Column Wash**" procedure, (Step **3**).

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Homogenization was incomplete	Depending on the sample, further vortexing with the flat bed vortex or bead beater equipment may be required. However, it is not recommended to increase the vortex time to longer than 10 minutes at maximum speed.
	Column has become clogged	Do not exceed the recommended input amount of 200 mg of stool. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels.
	An alternative Elution Buffer was used	It is recommended that the Elution Buffer E 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 A	Ensure that 90 mL of 96-100% ethanol is added to the supplied Wash Solution A prior to use.
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.
	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.
	DNase used may not be RNase-free	Ensure that the DNase being used for the optional On-Column DNA Removal step RNase-free, in order to prevent possible problems with RNA degradation.

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 A	Traces of salt from the binding step may remain in the sample if the column is not washed 3 times with Wash Solution A. 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.

Related Products	Product #
Stool DNA Isolation kit	27600
Total RNA Purification Kit	17200
1kb RNA Ladder	15003
HighRanger 1kb DNA Ladder	11900
UltraRanger 1kb DNA Ladder	12100
Stool Nucleic Acid Collection and Preservation Tubes	45660
Stool Nucleic Acid Collection and Preservation System	63700

Technical Support
Contact our Technical Support Team between the hours of 9:00 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

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
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Toll Free in North America: 1-866-667-4362

