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Stool Nucleic Acid Isolation Kit — Product Insert

Product 45600

Norgen's Stool Nucleic Acid Isolation kit provides a convenient and rapid method to isolate total DNA and RNA from fresh or frozen stool samples. The universal protocol conveniently allows for the isolation of total genomic DNA and total RNA from all the various microorganisms and host cells found in the stool sample simultaneously. The kit removes all traces of humic acid using the provided Bead Tubes and a combination of chemical and physical homogenization and lysis, without the use of phenol-chloroform extractions. A simple and rapid spin column procedure is then used to further purify the DNA and RNA. The purified DNA and RNA are of the highest quality and are fully compatible with downstream PCR applications, as all humic acid substances and PCR inhibitors are removed during the isolation.

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 vortexing for 3 minutes in order to efficiently and rapidly homogenize the sample and extract the DNA and RNA. The sample is then centrifuged, and the supernatant is transferred to a DNase and RNase-free microcentrifuge tube. Binding Buffer E is added, and the lysate is incubated for 10 minutes on ice. The lysate is then spun for 3 minutes to pellet any cell debris, the supernatant is collected, an equal volume of 70% ethanol is added to the lysate and the solution is loaded onto a spin-column. Norgen's resin binds nucleic acids in a manner that depends on ionic concentrations, thus only the DNA and RNA will bind to the column while the proteins are removed in the flowthrough or retained on top of the resin. The bound DNA and RNA are then washed using the provided Wash Solution A, and the purified nucleic acids are eluted using the Elution Buffer E. The purified total DNA and RNA are free of all inhibitors, including humic acid, and can be used in sensitive downstream applications including PCR.

Kit Components

Component	Product #45600 (50 preps)
Lysis Buffer C	60 mL
Binding Buffer E	6 mL
Wash Solution A	38 mL
Elution Buffer E	6 mL
Bead Tube	50
Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Advantages

- Universal method to detect microorganisms and host cell simultaneously in stool samples
- Rapid and convenient spin-column format
- No phenol:chloroform extractions
- Remove all humic acid from DNA and RNA
- Isolate high quality total DNA and RNA for sensitive downsteam applications

Specifications

Kit Specifications	
Maximum Stool Input	200 mg fresh or frozen stool
Maximum Column Binding Capacity	50 μg
Maximum Column Loading Volume	650 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 the Stool Nucleic Acid Isolation kit:

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

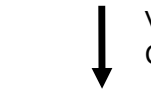
Working with RNA
RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended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

Flow Chart

Procedure for Purifying Total DNA and RNA using Norgen's Stool Nucleic Acid Isolation kit

Add stool sample and Lysis Buffer C to Bead Tube



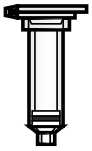
Vortex for 3 minutes.
Centrifuge. Transfer lysate.

Add Binding Buffer E.
Incubate for 10 minutes on ice.

SPIN

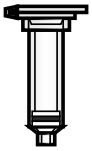
Transfer lysate

Add Ethanol



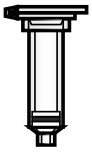
Bind to column

SPIN



Wash three times
with Wash Solution A.

SPIN



Elute RNA and DNA
with Elution Buffer E

SPIN

Purified Total RNA and DNA

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 **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 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.
- The maximum input of fresh or frozen stool sample is 200 mg. If the stool sample is in a suspension, an equivalent volume should be processed.

1. Lysate Preparation

a. Add up to 200 mg of stool sample to a provided Bead Tube and add 1 mL of **Lysis Buffer C**. Vortex briefly to mix stool and Lysis Buffer C.

   For stool samples that have been preserved using Norgen's Stool Nucleic Acid Collection and Transport Tubes (Cat# 45630 and 45660), add 400 µL of preserved sample to a provided Bead Tube and add 600 µL of Lysis Buffer C. Vortex briefly to mix stool and Lysis Solution.

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 3 minutes at maximum speed.
c. Centrifuge the tube for 3 minutes at **20,000 × g (~14,000 RPM)**.
d. Transfer up to 600 µL of supernatant to a DNase and RNase-free microcentrifuge tube (not provided).
e. Add 100 µL of **Binding Buffer E**, mix by inverting the tube a few times, and incubate for 10 minutes on ice.
f. Spin the lysate for 3 minutes to pellet any cell debris.
g. Using a pipette, transfer up to 650 µL of supernatant (avoid contacting the pellet with the pipette tip) into a 2 mL DNase and RNase-free microcentrifuge tube (not provided).
h. 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 650 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 the remaining volume of lysate mixture.

3. DNase Treatment (Optional)

Norgen's Stool Nucleic Acid Isolation Kit isolates total DNA and RNA. In case of need to purify only total RNA an optional **On-Column DNA Removal Protocol** is provided in Appendix A. 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

4. 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 steps **4a** and **4b** to wash column a second time.

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.

5. DNA and 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 2-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 2 additional minutes.

d. **(Optional):** An additional elution may be performed if desired by repeating steps **4b** and **4c** using 50 µL of **Elution Buffer E.** It is recommended to perform this elution into a second microcentrifuge tube (not provided). The total yield can be improved by an additional 20-30% when this second elution is performed.

6. Storage of DNA and RNA

The purified nucleic acids 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 Nucleic Acid Isolation Kit isolates total DNA and RNA. In case of need to purify only total RNA an optional **On-Column DNA Removal Protocol** is required. 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.

a. 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.

b. Perform the Total RNA Isolation Procedure up to and including "**Binding to Column**" (Steps 1 and 2).

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

d. Apply 100 µL of the RNase-free DNase I solution prepared in Step 3a to the column and centrifuge at 20,000 x g (~14 000 RPM) for 1 minute.

   Note: Ensure that the entire DNase I solution passes through the column. If needed, spin at 20,000 x g (~14 000 RPM) for an additional minute.

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

   Note: Ensure Step 3e is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

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

g. Without any further centrifugation, proceed directly to the second wash step in the "**Column Wash**" section (Step 4c).

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor DNA and RNA Recovery	Homogenization was incomplete	Depending on the type of stool, 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 5 minutes at maximum speed. Also, ensure that the maximum input of 200 mg of stool is not exceeded, as this may also cause incomplete homogenization.
	An alternative elution buffer was used	It is recommended that the Elution Buffer E supplied with this kit be used for maximum DNA and RNA recovery.
	Ethanol was not added to the lysate	Ensure that an equal amount of 70% 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.
The elution does not perform well in downstream applications	Eluted sample is brown	Ensure Binding Buffer E is added to the lysate and that it is incubated on ice for 10 minutes prior to spinning down the lysate. Avoid any contact with the pellet or surface residue when collecting the supernatant after the 5 minute spin during Sample Preparation.
	The nucleic acids were not washed three 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 three times with the provided 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.
	Binding Buffer E was not added to the lysate	Ensure that the Binding Buffer E is added to the lysate and that it is incubated on ice for 10 minutes prior to spinning down the lysate.
	PCR reaction conditions need to be optimized	Take steps to optimize the PCR conditions being used, including varying the amount of template, changing the source of Taq polymerase, looking into the primer design and adjusting the annealing conditions.

Related Products	Product #
Stool DNA Isolation kit	27600
Stool Total RNA Purification Kit	49500
Soil DNA Isolation Kit	26500
Fungi/Yeast Genomic DNA Isolation kit	27300
Blood DNA Isolation Mini Kit	46300
Water RNA/DNA Purification Kit	26400
HighRanger 1kb DNA Ladder	11900
UltraRanger 1kb DNA Ladder	12100

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

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