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Stool DNA Isolation Kit

Product Insert

Product # 27600

Norgen's Stool DNA Isolation Kit provides a convenient and rapid method to isolate total DNA from fresh or frozen stool samples. The kit can also be used to isolate DNA from stool samples preserved using Norgen's Stool Nucleic Acid Collection and Transport Tubes. The universal protocol conveniently allows for the isolation of total genomic DNA 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. A simple and rapid spin column procedure is then used to further purify the DNA. The purified DNA is of the highest quality and is 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 L to a provided Bead Tube and vortexing briefly to mix. Lysis Additive A is then added to the Bead Tube and the tube is vortexed in order to efficiently and rapidly homogenize the sample, extract the DNA and remove all humic acids. The sample is then centrifuged, and the supernatant is transferred to a DNAse-free microcentrifuge tube. Binding Buffer I is added, and the lysate is incubated for 10 minutes on ice. The lysate is then spun for 2 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 will bind to the column while the proteins are removed in the flowthrough or retained on top of the resin. The bound DNA is then washed using the provided Buffer SK and Wash Solution A, and the purified DNA is eluted using the Elution Buffer B. The purified total DNA is free of all inhibitors, including humic acid, and can be used in sensitive downstream applications including PCR.

Kit Components

Component	Product #27600 (50 preps)
Lysis Buffer L	60 mL
Lysis Additive A	6 mL
Binding Buffer I	7 mL
Buffer SK	30 mL
Wash Solution A	18 mL
Elution Buffer B	8 mL
Bead Tube	50
Mini Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

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

Advantages

- Universal method to detect microorganisms and host cell simultaneously in stool samples
- Rapid and convenient rapid spin-column format
- Remove all humic acid from DNA samples
- Isolate high quality total DNA for down steam applications

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.

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

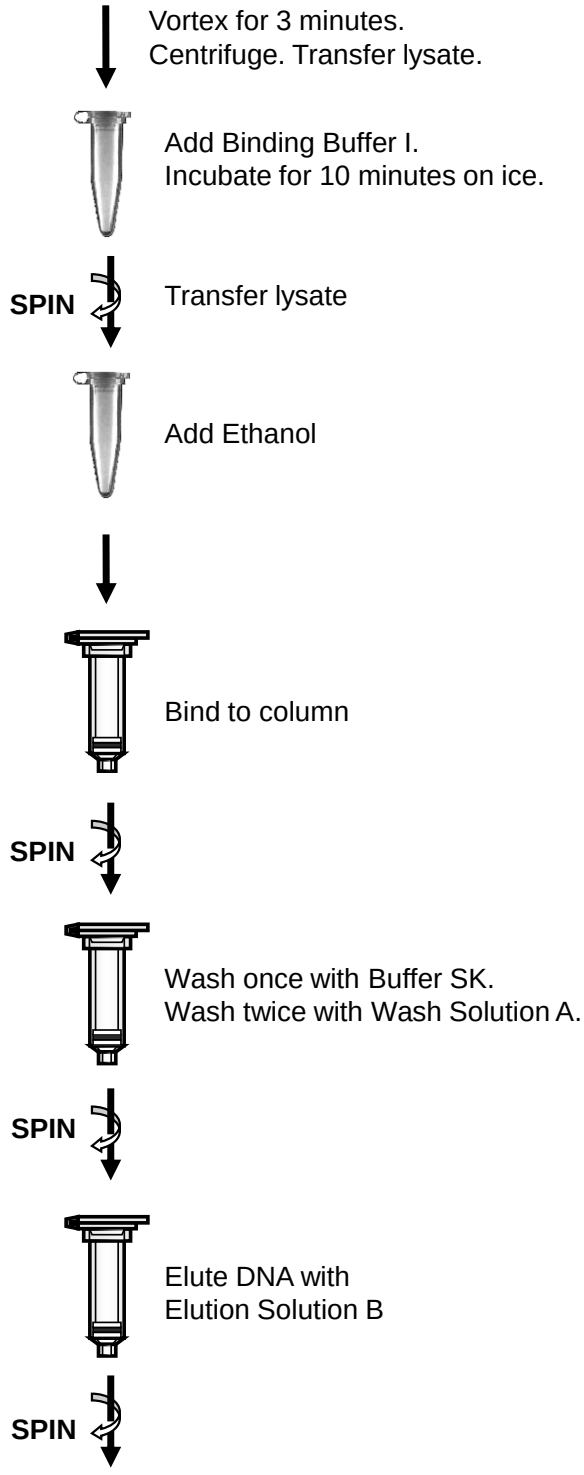
You must have the following in order to use the Stool DNA Isolation Kit:

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

Flow Chart

Procedure for Purifying Total DNA using Norgen's Stool DNA Isolation Kit

Add stool sample, Lysis Buffer L and Lysis Additive A to Bead Tube



Purified Total 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 **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 **Wash Solution A** by adding 42 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 60 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

1. Lysate Preparation

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

   For stool samples that have been preserved using Norgen's Stool Nucleic Acid Collection and Transport Tubes (Cat# 45650), add 200 µL of preserved sample to a provided Bead Tube and add 800 µL of **Lysis Buffer L**. Vortex briefly to mix stool and Lysis Solution.

b. Add 100 µL of **Lysis Additive A** and vortex briefly.
c. 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 minute at maximum speed.
d. Centrifuge the tube for 2 minute at **14000 × g (~14,000 RPM)**.
e. Transfer up to 600 µL of supernatant to a DNAase-free microcentrifuge tube (not provided).
f. Add 100 µL of **Binding Buffer I**, mix by inverting the tube a few times, and incubate for 10 minutes on ice.
g. Spin the lysate for 2 minutes to pellet any cell debris.
h. Using a pipette, transfer up to 700 µL of supernatant (avoid contacting the pellet with the pipette tip) into a 2 mL DNAase-free microcentrifuge tube (not provided).
i. 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 600 μL of the clarified lysate with ethanol onto the column and centrifuge for 1 minute at **≥ 3,500 x g (~6,000 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 at **14,000 x *g* (~14,000 RPM)**.

c. Repeat step **2b** with the remaining volume of lysate mixture.

3. Column Wash

a. Apply 500 μL of **Buffer SK** to the column and centrifuge for 1 minute.

Note: Ensure the entire Buffer SK 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. Apply 500 μL of **Wash Solution A** to the column and centrifuge for 1 minute.
d. Discard the flowthrough and reassemble the spin column with its collection tube.
e. Repeat **3c** and **3d**.
f. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

4. DNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 50 μL of **Elution Buffer B** to the column.
c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by a 1 minute spin at **14,000 x *g* (~14,000 RPM)**. Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at **14,000 x g (~14,000 RPM)** for 1 additional minute.
d. **(Optional):** An additional elution may be performed if desired by repeating steps **4b** and **4c** using 50 μL of Elution Buffer. The total yield can be improved by an additional 20-30% when this second elution is performed.

5. Storage of DNA

The purified genomic DNA can be stored at 2-8°C for a few days. For longer term storage, -20°C is recommended.

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor DNA 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 B supplied with this kit be used for maximum DNA recovery.
	Lysis Additive A was not added to the lysate	Ensure that the provided Lysis Additive A is added to separate humic acid and increase DNA yield. Also, an incubation can be preformed at 65°C for 10 minutes after addition of the Lysis Additive A and prior to vortexing to maximize DNA recovery.
	Ethanol was not added to the lysate	Ensure that an equal amount of ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution A	Ensure that 42 mL of 96 - 100% ethanol is added to the supplied Wash Solution A prior to use.
DNA does not perform well in downstream applications	Eluted DNA sample is brown	Ensure that the Lysis Additive A is added. Also ensure Binding Solution 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.
	Lysis Additive A was not added to the lysate	Ensure that the provided Lysis Additive A is added to the lysate.
	DNA was not washed with the provided Buffer SK and 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 Buffer SK and 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.

Problem	Possible Cause	Solution and Explanation
DNA does not perform well in downstream applications	Binding Buffer I was not added to the lysate	Ensure that the Binding Buffer I 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 Nucleic Acid Collection and Transport Tubes (50 Tubes)	45650
Stool Nucleic Acid Isolation Kit	45600
Stool Total RNA Purification Kit	49500

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.

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