Stool DNA Isolation Kit
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 Binding Buffer C 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

<table>
<thead>
<tr>
<th>Component</th>
<th>Product #27600 (50 preps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysis Buffer L</td>
<td>60 mL</td>
</tr>
<tr>
<td>Lysis Additive A</td>
<td>6 mL</td>
</tr>
<tr>
<td>Binding Buffer I</td>
<td>7 mL</td>
</tr>
<tr>
<td>Binding Buffer C</td>
<td>30 mL</td>
</tr>
<tr>
<td>Wash Solution A</td>
<td>18 mL</td>
</tr>
<tr>
<td>Elution Buffer B</td>
<td>8 mL</td>
</tr>
<tr>
<td>Bead Tube</td>
<td>50</td>
</tr>
<tr>
<td>Mini Spin Columns</td>
<td>50</td>
</tr>
<tr>
<td>Collection Tubes</td>
<td>50</td>
</tr>
<tr>
<td>Elution tubes (1.7 mL)</td>
<td>50</td>
</tr>
<tr>
<td>Product Insert</td>
<td>1</td>
</tr>
</tbody>
</table>
Specifications

<table>
<thead>
<tr>
<th>Kit Specifications</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Stool Input</td>
<td>200 mg fresh or frozen stool</td>
</tr>
<tr>
<td>Maximum Column Binding Capacity</td>
<td>50 µg</td>
</tr>
<tr>
<td>Maximum Column Loading Volume</td>
<td>650 µL</td>
</tr>
<tr>
<td>Time to Complete 10 Purifications</td>
<td>30 minutes</td>
</tr>
</tbody>
</table>

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.

Binding Buffer C contains guanidine hydrochloride and alcohol, 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 this solution.

Customer-Supplied Reagents and Equipment

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

- Benchtop microcentrifuge
- 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

1. Add stool sample, Lysis Buffer L and Lysis Additive A to Bead Tube
   - Vortex for 3 minutes.
   - Centrifuge. Transfer lysate.

2. Add Binding Buffer I.
   - Incubate for 10 minutes on ice.

3. Transfer lysate

4. Add Ethanol

5. Bind to column

6. Spin

7. Wash once with Binding Buffer C.
   - Wash twice with Wash Solution A.

8. Spin

9. Elute DNA with Elution Solution B

10. Spin

11. 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

- 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 Solution.

   For stool samples that have been preserved using Norgen’s Stool Nucleic Acid Collection and Transport Tubes (Cat# 45650), add 400 µL of preserved sample to a provided Bead Tube and add 600 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. OMNI BEAD RUPTOR). Vortex for 5 minutes at maximum speed for a flat-bed vortexer or S=5.00, T=0:20, D=0:10 and C=0.2 program on OMNI BEAD RUPTOR.

   d. Centrifuge the tube for 2 minutes at 14,000 RPM (~20,000 x g).

   e. Transfer up to 600 µL of clean supernatant to a DNAase-free microcentrifuge tube (not provided).

   Note: A thin layer will be formed on the top of the clean supernatant. Carefully collect only the clean supernatant although small pieces of the layer will not affect the quality of the DNA

   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 at 14,000 RPM (~20,000 x g) to pellet any cell debris.

   h. Using a pipette, transfer up to 500 µL of clean supernatant (avoid contacting the pellet or layer on the top of the supernatant) 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 14,000 RPM (~20,000 × g). 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 RPM (~20,000 × g).
   c. Repeat step 2b with the remaining volume of lysate mixture.

3. Column Wash
   a. Apply 500 μL of Binding Buffer C to the column and centrifuge for 1 minute at 10,000 RPM (~10,000 × g).
   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 at 10,000 RPM (~10,000 × g).
   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 at 10,000 RPM (~10,000 × g) 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 100 μL of Elution Buffer B to the column and incubate at room temperature for 1 minute.
   c. Centrifuge for 1 minute at 10,000 rpm (~10,000 x g).

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

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution and Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor DNA Recovery</td>
<td>Homogenization was incomplete</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>An alternative elution buffer was used</td>
<td>It is recommended that the Elution Buffer B supplied with this kit be used for maximum DNA recovery.</td>
</tr>
<tr>
<td></td>
<td>Lysis Additive A was not added to the lysate</td>
<td>Ensure that the provided Lysis Additive A is added to separate humic acid and increase DNA yield. Also, an incubation can be performed at 65°C for 10 minutes after addition of the Lysis Additive A and prior to vortexing to maximize DNA recovery.</td>
</tr>
<tr>
<td></td>
<td>Ethanol was not added to the lysate</td>
<td>Ensure that an equal amount of ethanol is added to the lysate before binding to the column.</td>
</tr>
<tr>
<td></td>
<td>Ethanol was not added to the Wash Solution A</td>
<td>Ensure that 42 mL of 96 - 100% ethanol is added to the supplied Wash Solution A prior to use.</td>
</tr>
<tr>
<td>DNA does not perform well in downstream applications</td>
<td>Eluted DNA sample is brown</td>
<td>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.</td>
</tr>
<tr>
<td></td>
<td>Lysis Additive A was not added to the lysate</td>
<td>Ensure that the provided Lysis Additive A is added to the lysate.</td>
</tr>
<tr>
<td></td>
<td>DNA was not washed with the provided Binding Buffer C and Wash Solution A</td>
<td>Traces of salt from the binding step may remain in the sample if the column is not washed three times with the provided Binding Buffer C and Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.</td>
</tr>
<tr>
<td></td>
<td>Ethanol carryover</td>
<td>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.</td>
</tr>
</tbody>
</table>
**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.

<table>
<thead>
<tr>
<th><strong>Problem</strong></th>
<th><strong>Possible Cause</strong></th>
<th><strong>Solution and Explanation</strong></th>
</tr>
</thead>
</table>
| 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**

<table>
<thead>
<tr>
<th>Related Products</th>
<th>Product #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool Nucleic Acid Collection and Transport Tubes (50 Tubes)</td>
<td>45650</td>
</tr>
<tr>
<td>Stool Nucleic Acid Isolation Kit</td>
<td>45600</td>
</tr>
<tr>
<td>Stool Total RNA Purification Kit</td>
<td>49500</td>
</tr>
<tr>
<td>Stool DNA Isolation Kit (Magnetic Bead System)</td>
<td>55700, 63100</td>
</tr>
</tbody>
</table>

**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