

## Product Information

### GenElute™ Stool DNA Isolation Kit

Catalog number **DNB200**

Storage: Room Temperature

## TECHNICAL BULLETIN

### Product Description

GenElute™ 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 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.

Purification is based on spin column chromatography using 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. The 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.

### Components

Materials Provided	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

### Reagents and Equipment Required But Not Provided.

- Benchtop microcentrifuge
- 1.7 mL DNase free micro centrifuge tubes
- DNase-free microcentrifuge tubes
- Flat bed vortex or bead beater equipment
- 96-100% ethanol

### Precautions and Disclaimer

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). This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Reagents to be prepared

Before beginning the procedure, prepare the following:

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.

### Storage/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.

### Procedure

#### Note:

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:

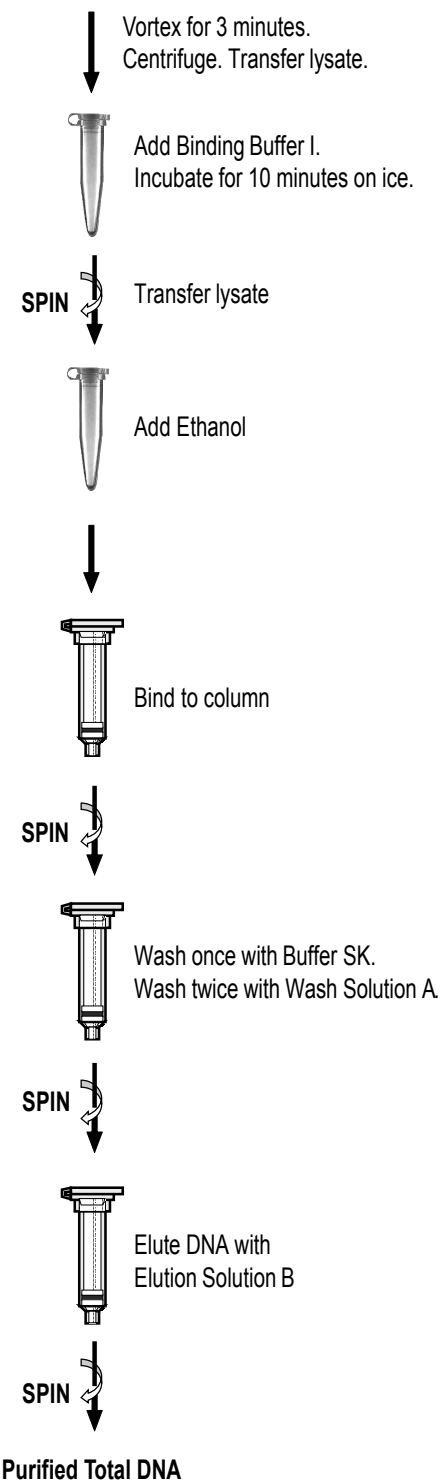
$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) (r)}}$$

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.

### Flow Chart

#### Procedure for Purifying Total DNA using GenElute™ Stool DNA Isolation Kit

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



Ensure that all solutions are at room temperature prior to use.

## Procedure

### 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.

**Note:** For stool samples that have been preserved using Stool Nucleic Acid Collection and Transport Tubes, add 200  $\mu$ L of preserved sample to a provided Bead Tube and add 800  $\mu$ L of Lysis Buffer L. Vortex briefly to mix stool and Lysis Solution.

- b. Add 100  $\mu$ L 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  $\times$  g (~14,000 RPM).
- e. Transfer up to 600  $\mu$ L of supernatant to a DNAase-free microcentrifuge tube (not provided).
- f. Add 100  $\mu$ L 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  $\mu$ L 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  $\mu$ L of ethanol is added to every 100  $\mu$ L 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  $\mu$ L of the clarified lysate with ethanol onto the column and centrifuge for 1 minute at  $\geq 3,500 \times g$  (~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  $\times$  g (~14,000 RPM).

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

### 3. Column Wash

- a. Apply 500  $\mu$ L 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  $\mu$ L 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  $\mu$ L of Elution Buffer B to the column.
- c. Centrifuge for 2 minutes at 200  $\times$  g (~2,000 RPM), followed by a 1 minute spin at 14,000  $\times$  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  $\times$  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  $\mu$ L 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.
	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.
DNA does not perform well in downstream applications	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.

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