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Soil DNA Isolation Kit — Product Insert

Product # 26500

Norgen's Soil DNA Isolation Kit provides a convenient and rapid method for the detection of microorganisms from soil samples. All types of soil samples can be processed with this kit, including common soil samples and difficult soil samples with high humic acid content such as compost 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 DNA. Total genomic DNA can be isolated and purified from all the various microorganisms found in soil, such as bacteria, fungi and algae. 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 soil sample and Lysis Solution to a provided Bead Tube and vortexing briefly to mix. Lysis Additive is then added to the Bead Tube and the tube is vortexed for 5 minutes 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 Solution is added, and the lysate is incubated for 5 minutes on ice. The lysate is then spun for 5 minutes to pellet any cell debris, the supernatant is collected, an equal volume of 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 Wash Solutions, and the purified DNA is eluted using the Elution Buffer. 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 # 26500 (50 preps)
Lysis Solution	45 mL
Lysis Additive	6 mL
Binding Solution	6 mL
Wash Solution I	30 mL
Wash Solution II	18 mL
Elution Buffer	6 mL
Bead Tubes	50
Mini Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50
Product Insert	1

Specifications

Kit Specifications	
Maximum Soil Input	250 mg
Type of Soil Processed	All types, including common soil, compost and manure
Column Binding Capacity	50 μg
Maximum Column Loading Volume	600 μL
Time to Complete 10 Purifications	30 minutes

Advantages

- Rapid and convenient method to detect microorganisms in soil samples
- Process all types of soil, including common soil, compost and manure
- Remove all humic acid from DNA samples
- Fast and easy processing using a rapid spin-column format
- Isolate high quality total DNA from a variety of microorganisms including bacteria, fungi and algae

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 Soil DNA Isolation Kit:

- Benchtop microcenrifuge
- DNAse-free microcentrifuge tubes
- Flat bed vortex or bead beater equipment
- 95-100% ethanol
- 70% ethanol

Flow Chart

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

Add soil sample, Lysis Solution and Lysis Additive to Bead Tube

Vortex for 5 minutes.
Centrifuge. Transfer lysate.

Add Binding Solution.
Incubate for 5 minutes.

SPIN

Transfer lysate

Add Ethanol

Bind to column

SPIN

Wash three times with
Wash Solutions

SPIN

Elute DNA with
Elution Buffer

SPIN

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 II** by adding 42 mL of 95 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution II**. 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 250 mg of soil sample to a provided Bead Tube and add 750 µL of **Lysis Solution**. Vortex briefly to mix soil and Lysis Solution.

   Note: In case of a wet soil sample, transfer the sample to a clean 1.7 mL microcentrifuge tube and centrifugation for 30 seconds at **14000 × g (~14,000 RPM**). Remove the water carefully using a pipette, and resuspend the soil pellet in 750 µL of **Lysis Solution.** Transfer the soil to a Bead Tube using a pipette. **Proceed to Step 1b.**

b. Add 100 µL of Lysis Additive 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 5 minutes at maximum speed.
d. Centrifuge the tube for 1 minute at **14000 × g (~14,000 RPM)**.
e. Transfer up to 400 µL of supernatant to a DNAase-free microcentrifuge tube (not provided).
f. Add 100 µL of Binding Solution, mix by inverting the tube a few times, and incubate for 5 minutes on ice.
g. Spin the lysate for 5 minutes to pellet any cell debris.
h. Using a pipette, transfer up to 450 µL of supernatant into a DNAase-free microcentrifuge tube (not provided).

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

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 up to 600 μL of the clarified lysate with ethanol onto the column and centrifuge for 1 minute at **14000 × 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. Depending on your lysate volume, repeat step **2b** if necessary.

3. Column Wash

a. Apply 500 μL of **Wash Solution I** 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. Apply 500 μL of **Wash Solution II** 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 100 μL of **Elution Buffer** 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 soil, 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 than10 minutes at maximum speed. Also, ensure that the maximum input of 250 mg of soil is not exceeded, as this may also cause incomplete homogenization.
	An alternative elution buffer was used	It is recommended that the Elution Buffer supplied with this kit be used for maximum DNA recovery.
	Lysis Additive was not added to the lysate	Ensure that the provided Lysis Additive is added to separate humic acid and increase DNA yield.
	Binding Solution was not added to the lysate	Ensure that the Binding Solution is added to the lysate and that it is incubated on ice for 5 minutes prior to spinning down the lysate.
	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	Ensure that 70 mL of 95 - 100% ethanol is added to the supplied Wash Solution prior to use.
DNA does not perform well in downstream applications	Eluted DNA sample is brown	Ensure that the Lysis Additive is added. Also, avoid any contact with the pellet or surface residue when collecting the supernatant after the 5 minute spin during Sample Preparation.
	Lysis Additive was not added to the lysate	Ensure that the provided Lysis Additive is added to the lysate. Also, an incubation can be preformed at 65°C for 10 minutes after addition of the Lysis Additive and prior to vortexing to maximize DNA recovery.
	DNA was not washed three times with the provided Wash Solutions	Traces of salt from the binding step may remain in the sample if the column is not washed three times with the provided Wash Solutions. 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.
	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 #
Plant/Fungi DNA Isolation kit	26200
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 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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