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Soil DNA Isolation Maxi Kit — Product Insert

Product # 62000

Norgen's Soil DNA Isolation Maxi Kit provides a convenient and rapid method for the detection of microorganisms from large soil samples (up to 10 grams). 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 OSR (Organic Substance Removal) Solution. 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 and NGS 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. The process involves first adding the soil sample, Lysis Buffer D and Lysis Additive A to a provided Bead B Tube, and the soil sample is homogenized. 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. This step is then to be repeated using the provided OSR (Organic Substance Removal) Solution to remove organic substances. The clean lysate is then collected and Lysis Buffer QP and ethanol are added. Next, the clean lysate is loaded onto a spin-column, which binds only the DNA. The bound DNA is then washed using the provided Binding Buffer B 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 and NGS.

Kit Components

Component	Product # 62000 (10 preps)
Lysis Buffer D	1 x 125 mL 1 x 45 mL
Lysis Additive A	50 mL
Binding Buffer I	25 mL
OSR Solution	12 mL
Lysis Buffer QP	88 mL
Binding Buffer B	110 mL
Wash Solution A	2 x 38 mL
Elution Buffer B	30 mL
Maxi Bead B Tubes	10
Maxi Spin Column	10
Elution tube	10
Product Insert	1

Advantages

- Rapid and convenient method to detect microorganisms in up to 10 g of soil samples
- Process all soil types
- Efficiently remove organic substances and humic acid using the OSR Solution
- 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

Specifications

Kit Specifications	
Maximum Soil Input	10 g
Type of Soil Processed	All soil types
Maximum Column Binding Capacity	1.5 mg
Average Yield from 10 g of soil*	30-50 µg
Time to Complete 10 Purifications	35 minutes (hands on time)

* Average yields will vary depending upon soil sample types

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 Safety Data Sheets (SDSs). These are available as convenient PDF files online at *www.norgenbiotek.com*.

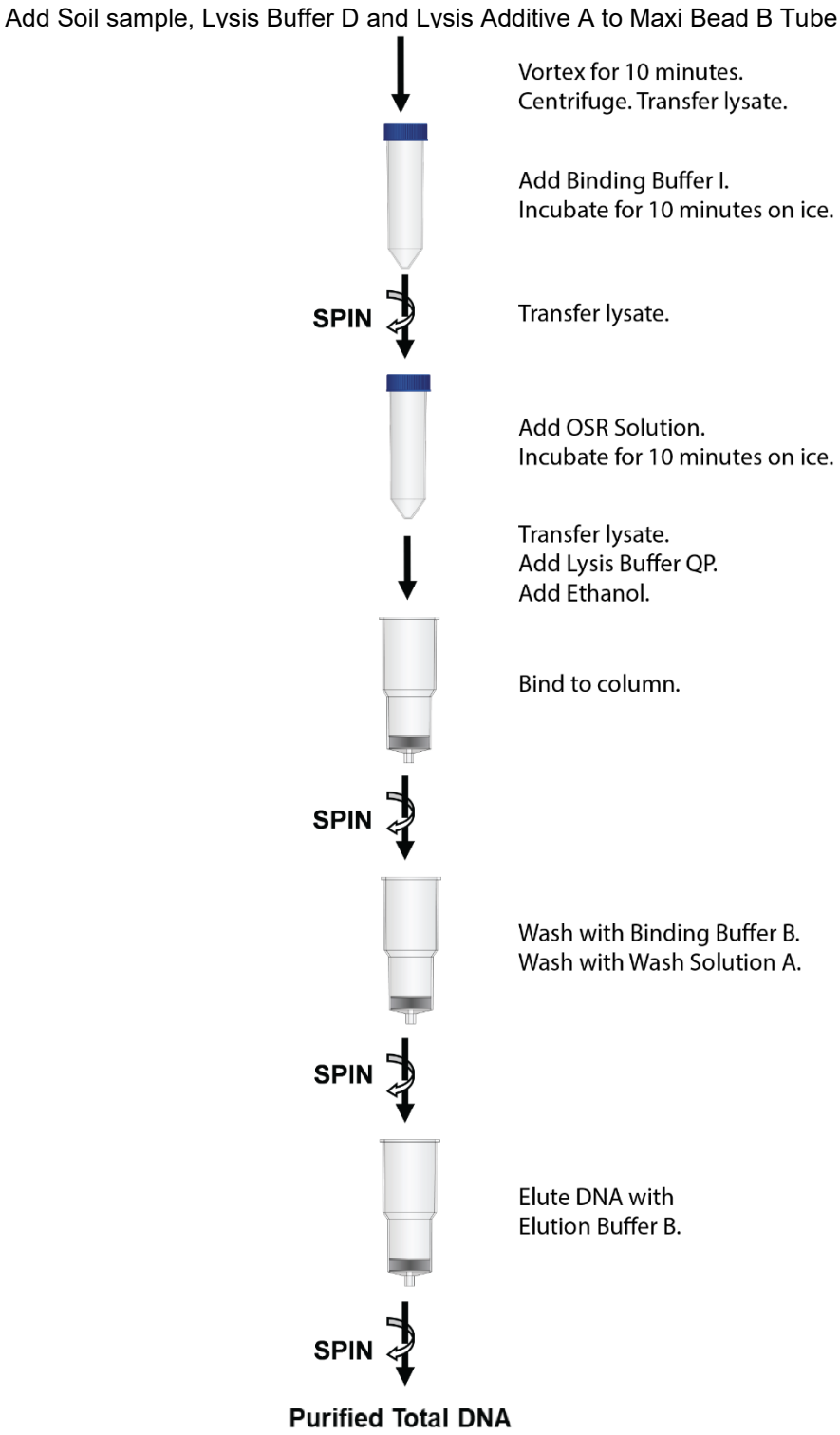
Customer-Supplied Reagents and Equipment

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

- Variable speed swing bucket centrifuge that can reach 2,500 x g (~4000 rpm) and can accommodate 50 mL centrifuge tubes
- 50 mL DNase free centrifuge tubes
- Flat bed vortex or bead beater equipment (e.g. Omni Bead Ruptor 50 mL Tube Carriage Kit)
- 96-100% ethanol

Flow Chart

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



Procedures

All centrifugation steps are carried out in a centrifuge with a swinging bucket. Various speeds are required for different steps, so please check your centrifuge 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

- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96-100% ethanol (provided by the user) to each of the supplied bottles containing 38 mL of concentrated **Wash Solution A**. This will give a final volume of 128 mL per bottle. The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.

1. Lysate Preparation

a. Add up to 10 grams of soil sample (see notes below) to a provided **Maxi Bead B Tube** and add 15 mL of **Lysis Buffer D.** Vortex briefly to mix the soil and Lysis Buffer D.

Note 1: Some soil samples, such as potting soil or top soil, will absorb the Lysis Buffer. Therefore, reduce the amount of soil input or increase the amount of **Lysis Buffer D** added up to 20 mL.

Note 2: In the case of a wet soil sample, transfer the sample to a clean 50 mL centrifuge tube and centrifugation for 30 minutes at **2,500 × g (~4,000 RPM)**. Remove the water carefully using a pipette, and resuspend the soil pellet in 15 mL of **Lysis Buffer D.** Transfer the soil to a Maxi Bead B Tube. **Proceed to Step 1b.**

b. Add 4 mL of **Lysis Additive A** and vortex briefly.

c. Secure the Maxi Bead B Tube horizontally on a flat-bed vortex pad with tape or secure the tube in any commercially available bead beater equipment. For a flat-bed vortexer, vortex for 10 minutes at maximum speed.

Note: If a proper vortexer is not available, alternatively incubate at 65°C for 30 minutes. Vortex the sample every 10 minutes for 10 seconds during the incubation.

d. Centrifuge the tube for 3 minutes at **2,500 x g (~4,000 RPM)** to pellet any soil particles. The color of the supernatant will be still turbid at this point.

e. Transfer clean supernatant to a DNase free 50 mL tube (not provided).

f. Add 2 mL of **Binding Buffer I**, mix by inverting the tube a few times, and incubate for 10 minutes on ice or 4 °C.

g. Spin the lysate for 5 minutes at **2,500 × g (~4,000 RPM)** to pellet any protein and soil particles.

h. Using a pipette, transfer up to 14 mL of clean supernatant into a DNase-free 50 mL tube (not provided) without any contact with the pellet.
i. Add 500 µL of **OSR Solution**, mix by inverting the tube a few times, and incubate for 10 minutes on ice or at 4˚C. Spin the lysate for 5 minutes at **2,500 × g (~4,000 RPM)**.
j. Transfer up to 14 mL of clean supernatant to a DNase-free 50 mL tube (not provided) without any contact with the pellet.
k. Add 8 mL of **Lysis Buffer QP** and 11 mL of **96-100% Ethanol** (user provided). Vortex briefly. **Proceed to Step 2.**

2. Binding to Column

a. Obtain a provided Spin Column assembled with a collection tube.
b. Vortex briefly to mix the lysate with **Lysis Buffer QP** and ethanol, and using a pipette apply the clarified lysate with ethanol (approximately 33 mL) onto the column and centrifuge for 2 minutes at **2,500 × g (~4,000 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. Discard the flowthrough. Reassemble the spin column with its collection tube.
d. Repeat Step 2b and 2c two more time until all the mixture has been transferred to the Maxi Spin column.

3. Column Wash

a. Apply 10 mL of **Binding Buffer B** to the column and centrifuge for 2 minutes at **2,500 × g (~4,000 RPM).**

   Note: Ensure the entire 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 10 mL of **Wash Solution A** to the column and centrifuge for 2 minutes at **2,500 × g (~4,000 RPM)**.
d. Discard the flowthrough and reassemble the spin column with its collection tube
e. Repeat Steps 3c and 3d for a second wash step.
f. Spin the column for 5 minutes at **2,500 x g (~4,000 rpm)** in order to thoroughly dry the resin. Discard the collection tube.

4. DNA Elution

a. Place the column into an elution tube provided with the kit.
b. Add 2 mL of **Elution Buffer B** to the column and incubate for 2 minutes at room temperature.
c. Centrifuge for 2 minutes at **2,500 × g (~4,000 RPM).**
d. **(Optional):** An additional elution may be performed if desired by repeating steps **4b** and **4c** using 2 mL of Elution Buffer B in a different elution tube (not provided). 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 may be required. However, it is not recommended to increase the vortex time to longer than 15 minutes at maximum speed.
	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.
	96-100% Ethanol was not added to the lysate	Ensure that 11 mL of 96 - 100% ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution A	Ensure that 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	The elution contains high humic acids. Ensure that the OSR Solution was added to the clean lysate.
	DNA was not washed with the provided Binding Buffer B and Wash Solution A	Traces of humic acids or salt from the binding step may remain in the sample if the column is not washed with the provided Binding Buffer B and Wash Solution A. Humic acids and 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 (10 ng to 20 ng for 20 µL of PCR reaction is recommended), changing the source of *Taq* polymerase, looking into the primer design and adjusting the annealing conditions.

Related Products	Product #
Soil DNA Isolation Plus kit	64000
Water RNA/DNA Purification Kit (0.22 µm)	26400
Water RNA/DNA Purification Kit (0.45 µm)	26450
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.

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