NORGEN BIOTEK CORPORATION

3430 Schmon Parkway
Thorold, ON, Canada L2V 4Y6
Phone: 866-667-4362 • (905) 227-8848
Fax: (905) 227-1061
Email: techsupport@norgenbiotek.com

Soil DNA Isolation Plus Kit — Product Insert

Product # 64000

Norgen's Soil DNA Isolation Plus 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 and PCR inhibitors using the provided 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 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 G and Lysis Additive A to a provided Bead 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 5 minutes at 4˚C or on ice. This step is then 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 solution 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.

Kit Components

Component	Product # 64000 (50 preps)
Lysis Buffer G	45 mL
Lysis Additive A	2 x 6 mL
Binding Buffer I	7 mL
OSR Solution	3 mL
Lysis Buffer QP	25 mL
Binding Buffer B	30 mL
Wash Solution A	18 mL
Elution Buffer B	8 mL
Bead Tubes	50
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 soil types
Maximum Column Binding Capacity	50 μg
Maximum Column Loading Volume	650 μL
Time to Complete 10 Purifications	30 minutes

Advantages

- Rapid and convenient method to detect microorganisms in soil samples
- Process all soil types
- Remove organic substances using the OSR Solution
- 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 Plus Kit:

- Benchtop microcenrifuge
- 1.7 mL DNase free micro centrifuge tubes
- DNAse-free microcentrifuge tubes
- Flat bed vortex or bead beater equipment(e.g. MP Biomedicals' FastPrep®-24 Instrument)
- 96-100% ethanol

Flow Chart

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

Add soil sample, Lysis Buffer G and Lysis Additive A to Bead Tube

Vortex for 5 minutes.
Centrifuge. Transfer lysate.

Add Binding Buffer I.
Incubate for 5 minutes on ice.

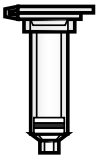
SPIN 

Transfer lysate.

Add OSR Solution.
Incubate for 5 minutes on ice.

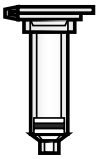
SPIN 

Transfer lysate
Add Lysis Buffer QP.
Add Ethanol.



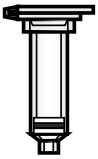
Bind to column

SPIN 



Wash with Binding Buffer B
Wash with Wash Solution A

SPIN 



Elute DNA with
Elution Buffer B

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

- 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 250 mg of soil sample (maximum input varies depending on the sample type) to a provided Bead Tube and add 750 µL of **Lysis Buffer G.** Vortex briefly to mix soil and Lysis Buffer G.

   Note: In the case of a wet soil sample, transfer the sample to a clean 1.7 mL microcentrifuge tube and centrifugation for 30 seconds at **20,000 × g (~14,000 RPM**). Remove the water carefully using a pipette, and resuspend the soil pellet in 750 µL of **Lysis Buffer G.** Transfer the soil to a Bead Tube using a pipette. **Proceed to Step 1b.**

b. Add 200 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. MP Biomedicals' FastPrep®-24 Instrument). Vortex for 30 seconds at 4M/S using a FastPrep®-24 instrument, or 5 minutes using a flat-bed vortexer at maximum speed. Alternatively, optimize the time and speed according to the manufacturer's manual.
d. Centrifuge the tube for 1 minute at **20,000 × g (~14,000 RPM)**.
e. Transfer clean supernatant to a DNase-free microcentrifuge tube (not provided) without any contact with the pellet.
f. Add 100 µL of **Binding Buffer I**, mix by inverting the tube a few times, and incubate for 5 minutes on ice or at 4˚C.
g. Spin the lysate for 2 minutes at **20,000 × g (~14,000 RPM)** to pellet any protein and soil particles.
h. Using a pipette, transfer up to 700 µL of clean supernatant into a DNase-free microcentrifuge tube (not provided) without any contact with the pellet.
i. Add 50 µL of **OSR Solution**, mix by inverting the tube a few times, and incubate for 5 minutes on ice or at 4˚C. Spin the lysate for 2 minutes at **20,000 × g (~14,000 RPM).**

j. Transfer up to 700 µL of clean supernatant to a DNase-free microcentrifuge tube (not provided) without any contact with the pellet.
k. Add 400 µL of **Lysis Buffer QP** and 550 µL of **96-100% Ethanol** (user provided). Vortex briefly. **Proceed to Step 2.**

2. Binding to Column

a. Assemble a provided Spin Column with one of the provided collection tubes.
b. Gently mix the lysate and **Lysis Buffer QP** using a pipette and apply 600 µL of the clarified lysate onto the column and centrifuge for 30 seconds at **10,000 x g (~10,000 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. Repeat Step 2b with the remaining lysate.

3. Column Wash

a. Apply 500 µL of **Binding Buffer B** to the column and centrifuge for 1 minute at **10,000 x g (~10,000 rpm)**.

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 A** to the column and centrifuge for 1 minute at **10,000 x g (~10,000 rpm)**.
d. Discard the flowthrough and reassemble the spin column with its collection tube.
e. Repeat step 3c and 3d.
f. Spin the column for 2 minutes at **20,000 x g (~14,000 rpm)** 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 50-100 µL of **Elution Buffer B** to the column and incubate for 1 minute at room temperature.
c. Centrifuge for 1 minute at **10,000 x g (~10,000 rpm)**.

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 than 10 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.
	Lysis Buffer QP and Ethanol were not added to the lysate	Ensure that 400 µL of Lysis Buffer QP and 550 µL of 96-100% Ethanol are 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	The elution contains high humic acids. Ensure that the OSR Solution was added to the clean lysate. Also ensure the column was washed with Binding Buffer B.
	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 50 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 #
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.

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362

