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 96-Well Kit (Slurry Format) Product Insert

Product # 43700

Norgen's Soil DNA Isolation 96-Well Kit (Slurry Format) provides a fast, reliable and simple procedure for high throughput isolation of DNA from 2 gram 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 Purifier and a combination of chemical and physical homogenization and lysis. A simple and rapid slurry 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. Typical yields of DNA isolated will vary depending on the input sample, with more fresh samples tending to yield more 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 using Norgen's proprietary resin as the separation matrix. The process involves first adding the soil sample, Lysis Solution and Lysis Additive to a provided Bead Tube and vortexing briefly to mix. The Bead Tube is vortexed for 5 minutes in order to efficiently and rapidly homogenize the sample and extract the DNA. The sample is then centrifuged, and the supernatant is transferred to a DNAse-free 5mL or 15 mL tube. Purifier is added, and the lysate is incubated for 5 minutes at -20°C (or 10 minutes on ice). The lysate is then spun for 5 minutes to pellet humic acid and any soil debris, and the entire supernatant is poured into a DNAse-free 15 mL tube. The Slurry Mixture (containing proprietary resin), Binding Buffer and an equal volume of 70% ethanol are added to the lysate and the resulting solution is mixed briefly. The resin pellet is collected by centrifugation, the supernatant is decanted, and the pellet is mixed with Wash Solution I. The resin is then loaded onto a 96 well filter plate. Norgen's resin binds nucleic acids in a manner that depends on ionic concentrations, thus only the DNA will be bound to the resin while other contaminants are removed in the flowthrough or retained on top of the resin. The bound DNA is then washed three times using the provided Wash Solution II, 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	Contents
Lysis Solution	3 x 100 mL
Lysis Additive	50 mL
Purifier	50 mL
Slurry Mixture	20 mL
Binding Solution	50 mL
Wash Solution I	40 mL
Wash Solution II	30 mL
Elution Buffer	20 mL
Bead Tube	96
96-Well Filter Plate	1
Adhesive Tape	1
96-Well Collection Plate	1
96-Well Elution Plate	1
Product Insert	1

Specifications

Kit Specifications	
Maximum Soil Input	2 grams
Type of Soil Processed	All types, including common soil, compost and manure
Slurry Binding Capacity	50 µg
Maximum 96-Well Loading Volume	400 µL
Time to Complete 96 Purifications	90 minutes

Advantages

- Fast and easy processing using a rapid 96-well format
- High throughput analysis with a high volume of soil (up to 2 grams)
- 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
- 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 96-Well Kit (Slurry Format):

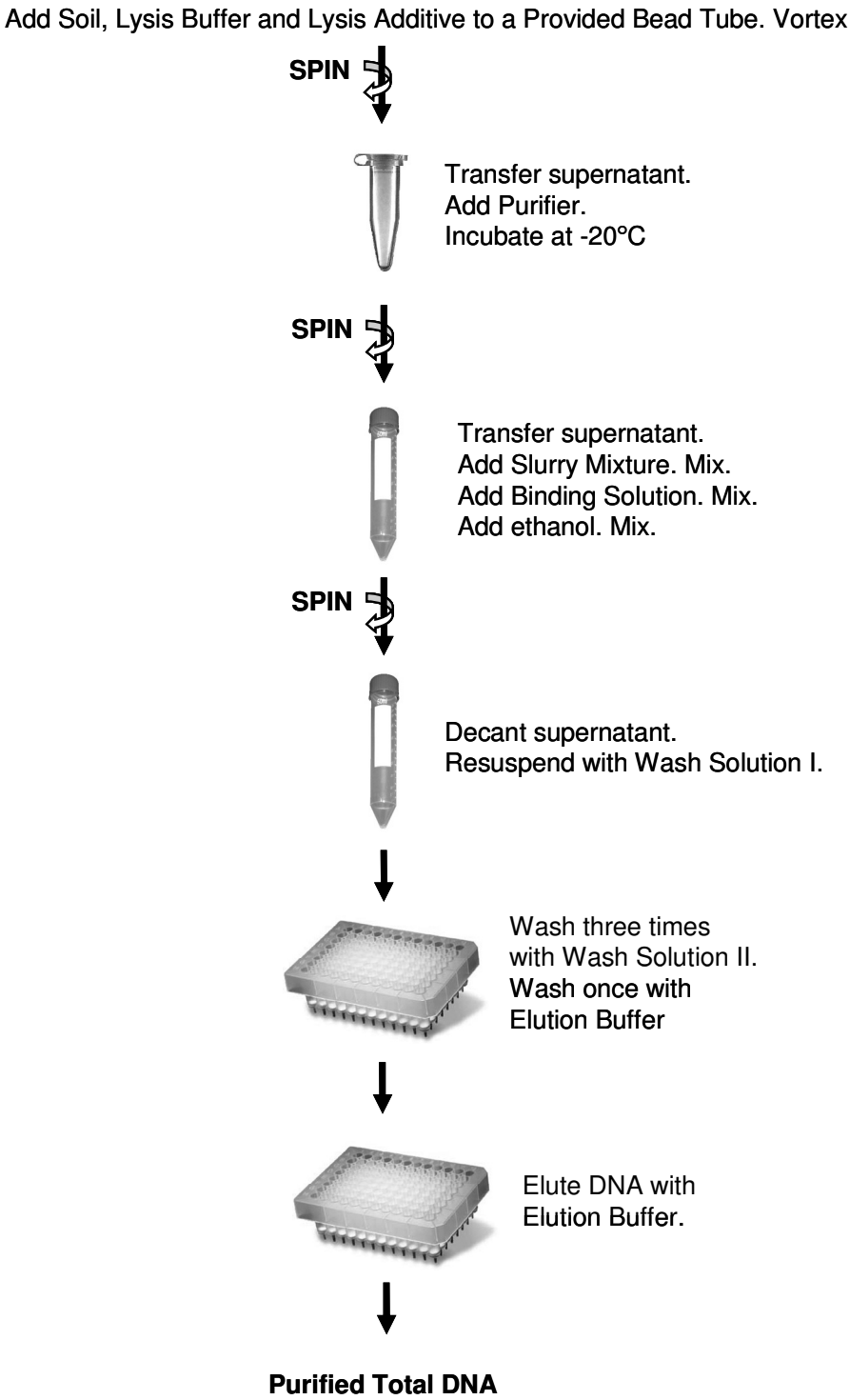
- Centrifuge with a swinging bucket rotor capable of 3000 x g
- -20°C freezer
- 15 mL tubes
- 2 mL microtube
- Flat bed vortex or bead beater equipment
- 95-100% ethanol
- 70% ethanol

- For ***Vacuum Format***:
  - Vacuum manifold with vacuum pump capable of generating a minimum pressure of -650 mbar or -25 in. Hg (such as Whatman UniVac 3 Vacuum to Collect Manifold)
  - Sealing tape or pads

- For ***Centrifuge Format***:
  - Centrifuge with rotor for 96-well plate assembly (such as Thermo Fisher IEC Centra CL3 series or Beckman GS-15R)

Flowchart

Procedure for Isolation of Soil DNA using Norgen's Soil DNA Isolation 96-Well Kit (Slurry Format)



Procedures

For *Vacuum Manifold*: All vacuum steps are performed at room temperature. The correct pressure can be calculated using the conversions:

1 **mbar** = 100 **Pa** = 0.0394 **in. Hg** = 0.75 **mm Hg** = 0.0145 **psi**

For *Centrifugation*: 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 II** by adding 90 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution II**. This will give a final volume of 120 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Slurry should be evenly mixed with the solution in the bottle prior to use.
- The maximum recommended input of soil sample is 2.0 g per well of the 96-Well Filter Plate.

1. Lysate Preparation

a. Add up to 2 g of soil sample to a provided **Bead Tube** and add 3 mL of **Lysis Solution**. Vortex briefly to mix soil and Lysis Solution.
b. Add 500 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. Vortex for 5 minutes at maximum speed or alternatively optimize the time and speed according to the manufacturer's manual.
d. Centrifuge the tube in a centrifuge with a swinging bucket rotor for 10 minutes at **3000 × g (~3000 RPM)**.
e. Transfer up to 1.5 mL of supernatant to a DNAase-free 2 mL microtube (not provided).
f. Add 500 µL of **Purifier**, mix by inverting the tube a few times, and incubate for 3 minutes at -20 ℃ (or 5 minutes on ice).
g. Spin the lysate for 5 minute at **14,000 × g (~14,000 RPM**) to pellet any protein and soil particles.
h. Pour entire supernatant into a DNAase-free 15 mL tube (not provided).
i. Add 200 µL of **Slurry Mixture** (Thoroughly resuspend the slurry by inverting the bottle several times prior to each pipetting). Vortex to mix.
j. Add 500 µL of **Binding Solution**. Vortex to mix.
k. Add an equal volume of 70% ethanol (provided by the user) to the lysate mixture above. Vortex for 30 seconds to mix.
l. Centrifuge the tube in a centrifuge with a swinging bucket rotor for 2 minutes at **3000 × g (~3000 RPM)** and decant entire supernatant.
m. Add 400 µL of **Wash Solution I** to the slurry pellet and mix well by pipeting.
n. **Proceed to Step 2.**

2. Total DNA Isolation

Note: The purification of total DNA from the lysate prepared in Section 1 could be performed using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in 2A. For purification using centrifugation, please follow the procedure outlined in 2B.

A. Total DNA Isolation Using Vacuum Manifold

2. **Binding DNA to 96-Well Filter Plate**

a. Assemble the 96-Well Filter Plate and the vacuum manifold according to manufacturer's recommendations.
b. Transfer 300 µL of the slurry mixture, including all slurry, into a well of the 96- Well Filter Plate.
c. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.
d. Turn off vacuum and ventilate the manifold. Discard the flowthrough. Reassemble the 96-Well Filter Plate and the vacuum manifold.
e. Repeat steps **2b**-**2d** with the remained slurry mixture.

Note: Ensure that all of the lysate from each well has passed through into the collection/waste tray. If the entire lysate volume has not passed, apply vacuum for an additional 2 minutes.

3. DNA Wash

a. Apply 400 µL of **Wash Solution II** to each well of the 96-Well Filter Plate. Tape the plate or any unused wells using sealing tape or pads (provided by the user) according to the vacuum manifold manufacturer's recommendations. Apply vacuum for 2 minutes.

Note: Ensure the entire wash solution has passed through into the collection/waste tray by inspecting the 96-Well Filter Plate. If the entire wash volume has not passed, apply vacuum for an additional 2 minutes.

b. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
c. Reassemble the 96-Well Filter Plate and the vacuum manifold. Repeat steps **3a** and **3b** to wash column for a second time.
d. Reassemble the 96-Well Filter Plate and the vacuum manifold. Repeat steps **3a** and **3b** to wash column for a third time.
e. Pat the bottom of the 96-Well Filter Plate dry. Reassemble the 96-Well Filter Plate and the vacuum manifold.
f. Perform a final wash by adding 75 µL of **Elution Buffer** to each well of the plate.
g. Apply vacuum for an additional 5 minutes.
h. Turn off vacuum, ventilate the manifold and discard the flowthrough.

4. DNA Elution

a. Replace the collection/waste tray in the vacuum manifold with the provided Elution Plate. Complete the vacuum manifold assembly with the 96-Well Filter Plate.
b. Add 100 µL of **Elution Buffer** and apply vacuum for 2 minutes.
c. Turn off vacuum and ventilate the manifold.

Note: As a template for any downstream application, 3-6 ng of DNA from the elution is recommended.

5. Storage of DNA

Use the provided adhesive tape to seal the Elution Plate. The purified DNA samples may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

B. Total DNA Purification Using Centrifugation

Note: To purify total DNA using a vacuum manifold please follow Section A above.

2. Binding DNA to 96-Well Filter Plate

a. Place the 96-Well Filter Plate on top of a provided 96-Well Collection Plate.
b. Transfer 300 µL of the slurry mixture into a well of the 96- Well Filter Plate.
c. Centrifuge the assembly at 3,000 *x g* (~3,000 RPM) for 2 minutes.
d. Discard the flowthrough. Reassemble the 96-Well Filter Plate and the Collection plate.
e. Repeat steps **2b**-**2d** with the remained slurry mixture.

Note: Ensure that all of the lysate from each well has passed through into the collection plate. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.

3. DNA Wash

a. Apply 400 µL of **Wash Solution II** to each well of the 96-Well Filter Plate. Centrifuge the assembly 3,000 *x g* (~3,000 RPM) for 2 minutes.

Note: Ensure the entire wash solution has passed through into the Collection plate by inspecting the 96-Well Filter Plate. If the entire wash volume has not passed, centrifuge for an additional 2 minutes.

b. Discard the flowthrough. Reassemble the 96-Well Filter Plate and the bottom plate.
c. Repeat steps **3a** and **3b** to wash column for a second time.
d. Repeat steps **3a** and **3b** to wash column for a third time.
e. Pat the bottom of the 96-Well Filter Plate dry. Reassemble the 96-Well Filter Plate and the bottom plate.
f. Perform a final wash by adding 75 µL of **Elution Buffer** to each well of the 96-Well Filter Plate. Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 10 minutes.
g. Discard the flowthrough.

4. DNA Elution

a. Stack the 96-Well Filter Plate on top of the provided Elution Plate.
b. Add 100 µL of **Elution Buffer** to each well of the 96-Well Filter Plate.
c. Centrifuge the assembly at maximum speed or 3,000 *x g* (~3,000 RPM) for 2 minutes.

Note: As a template for any downstream application, 3-6 ng of DNA from the elution is recommended.

5. Storage of DNA

Use the provided adhesive tape to seal the Elution Plate. The purified DNA samples may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

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 was not added to the lysate	Ensure that the provided Lysis Additive is added to increase DNA yield.
	70% Ethanol was not added to the lysate	Ensure that an equal amount of 70% ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution II	Ensure that 90 mL of 95 - 100% ethanol is added to the supplied Wash Solution II prior to use.
DNA does not perform well in downstream applications	Eluted DNA sample is brown	Reduce the volume of soil input. Also repeat the purifying step 1f and 1g.
	Purifier was not added to the lysate	Ensure that the provided Purifier is added to the lysate to remove humic acid.
	DNA was not washed three times with the provided Wash Solutions	Traces of humic acids or salt from the binding step may remain in the sample if the column is not washed three times with the provided Wash Solutions. Humic acids and salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the final wash under the Column Wash procedure is performed with 75 µL of Elution Buffer, 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 (3 ng to 6 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 #
Plant/Fungi DNA Isolation kit	26200
Water RNA/DNA Purification Kit	26400
Soil DNA Isolation kit	26500
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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