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Soil DNA Isolation 96 Well Kit — Product Insert

Product # 26560

Norgen's Soil DNA Isolation 96-Well Kit provides a fast, reliable and simple procedure for high throughput isolation of DNA from all types of soil samples including common soil samples and difficult soil samples with high humic acid content such as compost and manure. A combination of chemical and physical homogenization effectively lyses all microorganisms in the soil sample. The kit removes all traces of humic acid using the provided Organic Substance Removal (OSR) Solution. 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 for any metagenomic study, as all humic acid substances and PCR inhibitors are removed during the isolation.

Norgen's Purification Technology

Purification is based on a 96-well column chromatography. The DNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The purification could be performed on either a vacuum manifold or using centrifugation. 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 then added to the lysate, and it is incubated for 5 minutes on ice. This step is then to be repeated using the provide Organic Substance Removal (OSR) Solution for soil samples 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 96-Well Plate, 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 a number of downstream applications for metagenomic studies.

Kit Components

Component	Product # 26560 (192 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
Bead B Tubes	200
96-Well Plate	2
96-Well Filter Plate	2
96-Well Collection Plate	2
Adhesive Tape	4
96-Well Elution Plate	2
Product Insert	1

Advantages

- Fast and easy high throughput processing using either a vacuum manifold or centrifugation
- Process all soil types
- Remove organic substances and humic acid using the OSR Solution
- Isolate high quality total DNA from all soil types
- No phenol or chloroform extractions

Specifications

Kit Specifications	
Binding Capacity Per Well	50 μg
Maximum Loading Volume Per Well	500 μL
Size of DNA Purified	All sizes
Maximum Amount of Starting Material	250 mg
Time to Complete 96 Purifications	90 minutes

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 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*.

Lysis Buffer D, Binding Buffer B and **Lysis Buffer QP** contains guanidinium salts and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Customer-Supplied Reagents and Equipment

You must have the following in order to use the Soil DNA Isolation 96-Well Kit:

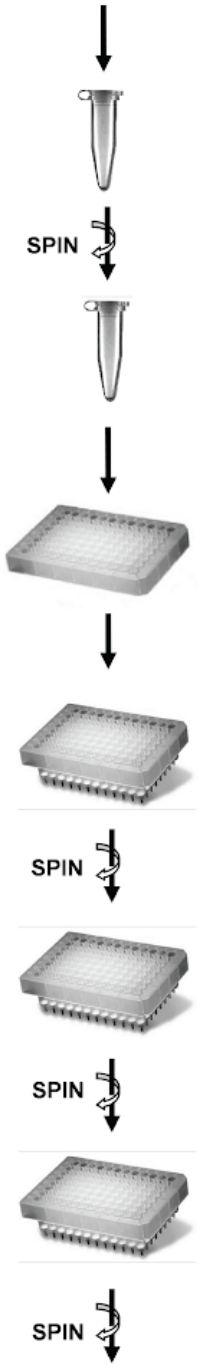
- Micropipettors and multichannel pipettes
- 96%-100% ethanol
- Ice bath
- Collection/Waste Tray for vacuum manifold or 96-well bottom plate (96-well format) for centrifugation
- DNase-free microcentrifuge tubes
- Flat bed vortex or bead beater equipment (e.g. MP Biomedicals' FastPrep®-24 instrument)
- For ***Vacuum Format***:
    - Vacuum manifold with vacuum pump capable of generating a minimum pressure of -60 kpa or -15 in. Hg (such as PALL Life Sciences Multi-Well Plate Vacuum 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 Purifying Soil DNA using Norgen's Soil DNA Purification 96-Well Kit

Add Soil sample, Lysis Buffer D and Lysis Additive A to Bead B Tube



Vortex for 10 minutes.
Centrifuge. Transfer lysate.

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

Transfer Lysate.

Add OSR Solution.
Incubate for 5 minutes on ice.

Transfer lysate to 96-Well plate.
Add Lysis Buffer QP.
Add Ethanol and mix.

Bind to 96-Well Filter Plate

Wash with Binding Buffer B
Wash with Wash Solution A

Elute DNA with Elution Buffer B

Purified Total DNA

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

- The isolation of DNA from soil can be performed using either a vacuum manifold or centrifugation. For purification using vacuum, please follow the procedure outlined in Section 2A. For purification using centrifugation, please follow the procedure outlined in Section 2B. Lysate preparation is the same for both procedures.
- Ensure that all solutions are at room temperature prior to use. If necessary, warm to 65°C to redissolve any precipitates.
- Prepare a working concentration of **Wash Solution A** by adding 90 mL of 96 - 100% ethanol (to be provided by the user) to each supplied bottle containing concentrated **Wash Solution A**. This will give a final volume of 128 mL. The labels on the bottles have a box that can be checked to indicate that ethanol has been added.
- The maximum recommended input of soil is 250 mg.
- The input volume however can be modified depending on user's decision based on the soil type. However, it is not recommended that more than 250 mg is used per well.

Section 1. Lysate Preparation

a. Add 250 mg of soil sample to a provided **Bead B Tube** and add 750 µL of **Lysis Buffer D.** Vortex briefly to mix soil and **Lysis Buffer D.**

   Note: In the case of a wet soil sample, transfer the sample to a clean 1.7 mL microcentrifuge tube and centrifuge 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 D.** Transfer the soil to a **Bead B Tube** using a pipette. **Proceed to Step 1b.**

b. Add 200 µL of **Lysis Additive A** and vortex briefly.
c. Secure bead tube to a vortex adapter and vortex at maximum speed for 10 minutes.

Alternatively, 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 96-Well plate (provided) without any contact with the pellet.
k. Add 400 µL of **Lysis Buffer QP** and 550 µL of **96-100% Ethanol** (user provided). Using a multichannel pipette, mix the lysate at least 10 times. **Proceed to Section 2.**

Section 2. 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 section 2A. For purification using centrifugation, please follow the procedure outlined in section 2B.

A. DNA Isolation Using Vacuum Manifold

1. Binding DNA to Filter Plates

Assemble the 96-Well Filter plate with a new 96-Well Collection Plate (provided) and the vacuum manifold according to manufacturer's recommendations.

a. Gently mix the lysate using a multichannel pipette and apply up to 600 µL of the lysate into each well of the 96-Well Filter Plate. Tape the plate or any unused wells using sealing tape or pads (not provided). Apply vacuum for 2 minutes.

   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.

b. Repeat step a two more times to apply the rest of the lysate into each well of the 96-Well Filter Plate.

2. DNA Wash

a. Apply 500 µL of **Binding Buffer B** to each well of the 96-Well Filter Plate. Tape the plate or any unused wells using sealing tape or pads (not provided). 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.
d. Apply 500 µL of **Wash Solution A** to each well of the 96-Well Plate. Tape the plate or any unused wells (not provided) and 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.

e. Turn off vacuum and ventilate the manifold. Discard the flowthrough.
f. Repeat steps d and e.
g. Reassemble the 96-Well Filter Plate and the vacuum manifold. Apply vacuum for an additional 10 minutes in order to completely dry the plate.
h. Turn off vacuum and ventilate the manifold.
i. Gently pat the bottom of the 96-well filter plate on a clean paper towel to remove residual wash solution.

3. 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 B** to each well of the plate.
c. Apply vacuum for 3 minutes.

4. 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. DNA Purification Using Centrifugation

Note: To purify total DNA using a vacuum manifold please follow Section 2A above.

1. Binding DNA to Filter Plates

a. Place the 96-Well Filter Plate on top of a new 96-Well Collection Plate (provided).
b. Gently mix the lysate using a multichannel pipette and apply up to 600 µL of the lysate into each well of the 96-Well Filter plate. Centrifuge the assembly at maximum speed or 4,000 x g (~4,000 RPM) for 2 minutes. Discard the flowthrough.
c. Reassemble the 96-Well Filter Plate and the 96-Well Collection Plate.
d. Repeat step b and c two more times to apply the rest of the lysate into each well of the 96-Well Filter Plate.

2. DNA Wash

a. Apply 500 µL of **Binding Buffer B** to each well of the 96-Well Filter Plate. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 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 collection plate.
c. Apply 500 µL of **Wash Solution A** to each well of the 96-Well Filter Plate. Centrifuge the assembly at maximum speed or 4,000 x g (~4,000 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.

d. Discard the flowthrough. Reassemble the 96-Well Filter Plate and the collection plate.
e. Repeat steps c and d.
f. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 10 minutes in order to completely dry the plate.
g. Gently pat the bottom of the 96-Well Filter plate on a clean paper towel to remove residual wash solution.

3. DNA Elution

a. Stack the 96-Well Filter Plate on top of the 96-Well Elution Plate.
b. Add 100 µL of **Elution Buffer B** to each well of the 96-Well Filter Plate and incubate for 1 minute at room temperature.
c. Centrifuge the assembly at maximum speed or 4,000 *x g* (~4,000 RPM) for 3 minutes.

4. 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, optimization of the homogenization 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.

	96-100% Ethanol was not added to the lysate	Ensure that 550 µL 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 90 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 to remove humic acids.
	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 Wash procedure is performed and pat the bottom of the 96-Well Filter plate on a clean paper towel to remove the residual wash solution. 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
Soil DNA Isolation Kit (Magnetic Bead System)	58100
Soil DNA Isolation Kit (High-throughput Magnetic Bead System)	62800

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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