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Soil DNA Isolation 96-Well Kit (Magnetic Bead System) Product Insert

Product # 62800

Norgen's Soil DNA Isolation 96-Well Kit (Magnetic Bead System) provides a fast and reproducible high - throughput method for isolating genomic DNA 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 the OSR (Organic Substance Removal) 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, as all humic acid substances and PCR inhibitors are removed during the isolation. Norgen's Soil DNA Isolation 96-Well Kit (Magnetic Bead System) can also be integrated with a robotic automation system.

Norgen's Purification Technology

Purification is based on the use of magnetic beads that bind DNA under optimized DNA binding conditions. Soil samples are first mixed with Lysis Buffer G and Lysis Additive A in the provided bead tube and homogenized. The clean lysate is then separated by centrifugation, followed by the addition of Binding Buffer I and incubation on ice for 5 minutes. This step can then be repeated using the provided OSR (Organic Substance Removal) Solution for soil samples containing high amounts of organic substances as an optional step. The lysate is then spun in order to remove any debris, and the lystate is then transferred into a well of the 96-well plate. Magnetic Bead Suspension and ethanol are then added to the clean supernatant, and the resulting solution is placed on the magnetic separation rack. Only the DNA will bind to the magnetic beads, while most of the proteins will be removed in the supernatant. The bound DNA is then washed with Buffer SK and 70% ethanol in order to remove any remaining impurities, and the purified total DNA is eluted with Elution Buffer B. The purified DNA can be used in a number of downstream applications.

Specifications

Kit Specifications	
Maximum Soil Input	0.25 g of all soil types
Average Yield from 0.25 mL of Soil*	1 - 5 μg
Average Purity (OD260/280)	1.7 – 1.8
Time to Complete 96 Purifications	50 minutes

* Average DNA yield will vary depending on the sample

Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature (15 – 25°C). These reagents should remain stable for at least 2 years in their unopened containers.

Advantages

- Fast and easy processing using a magnetic bead system
- Robust lysis system (chemical lysis combined with mechanical homogenization)
- Isolate high quality genomic DNA
- Consistent, high yields of inhibitor-free DNA up to 50 kb plus
- Isolate sequencing-quality total DNA from a variety of microorganisms including bacteria, fungi and algae
- High throughput and compatible with an automation robotic system

Kit Components

Component	Product #62800 (192 samples)
Lysis Buffer G	1 x 100 mL 1 x 45 mL
Lysis Additive A	25 mL
Binding Buffer I	25 mL
OSR Solution	12 mL
Binding Buffer B	85 mL
Magnetic Bead Suspension	8.5 mL
Buffer SK	60 mL
Elution Buffer B	15 mL
Bead Tubes	196
Adhesive Tape	2
96-Well Plate	2
96-Well Elution Plate	2
Product Insert	1

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*.

OSR Solution, Binding Buffer B and Buffer SK contain guanidinium salts, and should be handled with care. Guanidinium salts forms 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

- Magnetic bead separation plate
- Multi-channel Micropipettors
- Microcentrifuge tube
- Flat bed vortex or bead beater equipment(e.g. MP Biomedicals' FastPrep®-24 Instrument)
- 70% ethanol (prepare fresh)
- 96-100% ethanol
- Ice

Procedure

Notes prior to use:

- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- **Always** vortex the **Magnetic Bead Suspension** before use.

Flow Chart

Procedure for Purifying Soil DNA using
Norgen's Soil DNA Isolation 96-Well Kit (Magnetic Bead System)

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

SPIN

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

SPIN

OPTIONAL: Add OSR Solution.
Incubate on ice for 5 minutes.

Transfer supernatant. Add Binding Buffer B and ethanol. Mix. Add Magnetic Bead Suspension. Mix. Incubate for 5 minutes.

Place onto magnetic plate.
Let stand for 1 minute.

Discard supernatant. Add Buffer SK, mix and incubate for 1 minute.

Place onto magnetic plate.
Let stand for 1 minute.

Discard supernatant. Add 70% ethanol, mix and incubate for 1 minute.

Repeat ethanol wash step.
Incubate open tube at 65˚C for 5 minutes.

Add Elution Buffer B, mix and Incubate at 65˚C for 10 minutes.

Place onto magnetic plate.
Let stand for 1 minute.

Carefully transfer supernatant to Elution Plate.

Pure Soil DNA

1. Soil Sample Collection and Lysate Preparation

a. Add up to 250 mg of soil sample 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 100 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 25 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 2 minutes at 20,000 × g (~14,000 RPM).
e. Transfer up to 450 µL of supernatant to a DNAase-free microcentrifuge tube (not provided).
f. Add 100 µL of **Binding Buffer I**, mix by inverting the tube a few times and incubate for 5 minutes on ice or -20°C.
g. Spin the lysate for 2 minutes to pellet any protein and soil particles.

Note: For regular soil samples, proceed directly to Step i. For samples that are known to contain high amounts of organic substances, please proceed with the optional Step h below

h. **OPTIONAL Step for Soil Samples Containing High Amounts of Organic Substances:** Using a pipette, transfer up to 450 µL of supernatant into a DNase-free microcentrifuge tube (not provided) without any contact with the pellet. Add 50 µL of **OSR Solution**, mix by brief vortexing, and incubate for 5 minutes on ice. Spin the lysate for 2 minutes at 20,000 × g (~14,000 RPM) to pellet any protein and soil particles. Proceed to Step i.

i. Using a pipette, transfer up to 450 µL of supernatant (avoid contacting the pellet with the pipette tip) into a 96-Well Plate.
j. Add 400 µL **Binding Buffer B** and 425 µL **96-100% ethanol** (provided by the user). Mix by vortexing or pipetting.
k. Add 40 µL of **Magnetic Bead Suspension** (vortex prior to use) to the lysate collected above. Mix by vortexing or pipetting.
l. Incubate at room temperature for 5 minutes. Occasionally shake the 96-Well Plate.
m. Proceed to Section 2: **Soil DNA isolation**

2. Soil DNA Isolation

a. Place the 96-Well Plate on the magnetic plate. Allow to sit for 1 minute.
b. Aspirate and discard supernatant without touching the magnetic beads.
c. Remove the 96-Well Plate from the magnetic plate and gently add 500 µL of **Buffer SK**. Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute.
d. Place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute.
e. Aspirate and discard supernatant without touching the magnetic beads.
f. Remove the 96-Well Plate from the magnetic plate and gently add 500 µL of freshly prepared **70% ethanol**. Resuspend by pipetting and incubate at room temperature for 1 minute.
g. Place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute.
h. Aspirate and discard supernatant without touching the magnetic beads.
i. Repeat **Steps 2f - 2h** for a second wash step.

Note: Remove as much of the 70% ethanol in the sample plate as possible by pipetting.

j. Incubate the 96-Well Plate at 65˚C for 5 minutes to dry the magnetic beads.
k. Add 75 µL of **Elution Buffer B**. Mix by vortexing or pipetting and incubate at 65˚C for 10 minute.
l. Briefly vortex or pipette to mix and place the 96-Well Plate on the magnetic plate and let it sit for 1 minute.

m. Carefully transfer the elution to a 96-Well Elution Plate (provided) without touching the magnetic beads. The purified DNA sample may be stored at 4°C for a few days. The provided adhesive tape can be used for the storage of the DNA. It is recommended that samples be placed at –20°C for long-term storage

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Magnetic beads were accidently pipetted up with the supernatant.	The pipette tip was placed too close to the magnetic beads while pipetting	Return the magnetic beads and the supernatant back into the sample well. Mix well, and place the plate back onto the magnetic separation plate for the specified time. Carefully remove the supernatant without touching the magnetic beads.
The yield of genomic DNA is low	Incomplete lysis of cells	Ensure that Lysis Additive A is added. Also incubation at 65ºC may result in increased yields.
	Amount of magnetic beads added was not sufficient	Ensure that the magnetic bead suspension is mixed well prior to use to avoid any inconsistency in DNA isolation.
	DNA concentration in the soil sample being used is low.	Some soil type contain very little target DNA. Incubation at 65ºC may result in increased yields.
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 Buffer SK	Traces of humic acids or salt from the binding step may remain in the sample if the Magnetic beads are not washed with the provided Buffer SK. Humic acids and salt may interfere with downstream applications, and thus must be washed from the magnetic beads.
	Ethanol carryover	Ensure that the drying step after the 70% ethanol wash steps is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
RNA is present in eluted DNA.	RNA is coeluted with the DNA.	Carry out a digestion with RNase A on the elution if the RNAse present will interfere with downstream applications. Refer to manufacturer's instructions regarding amount of enzyme to use, optimal incubation time and temperature.

Related Products	Product #
Soil DNA Isolation Kit (Magnetic Bead System)	58100
Soil DNA Isolation Kit (50 Prep)	27600
Soil Nucleic Acid Isolation Kit	45600

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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