

Soil DNA Isolation Kit (Magnetic Bead System) – 50 Preps
Product # 58100

Product Insert

Norgen’s Soil DNA Isolation Kit (Magnetic Bead System) provides a fast and reproducible 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 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. 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	
Number of Preps	50
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 12 Purifications	40 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. This kit is stable for 1 year after the date of shipment.

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

Kit Components

Component	Product # 58100 (50 samples)
Lysis Buffer G	100 mL
Lysis Additive A	6 mL
Binding Buffer I	7 mL
OSR Solution	3 mL
Binding Buffer B	2 x 12 mL
Magnetic Bead Suspension	2 x 1.1 mL
Buffer SK	30 mL
Elution Buffer B	8 mL
Bead Tubes	50
Elution tubes (1.7 mL)	50
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 rack
- 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

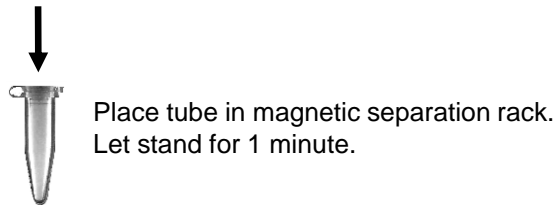
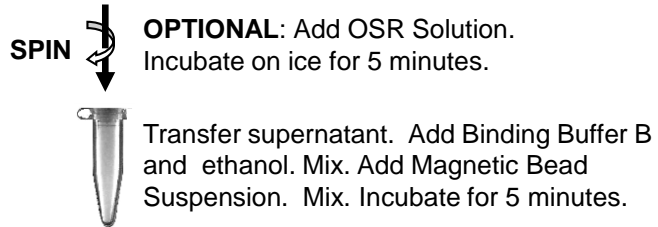
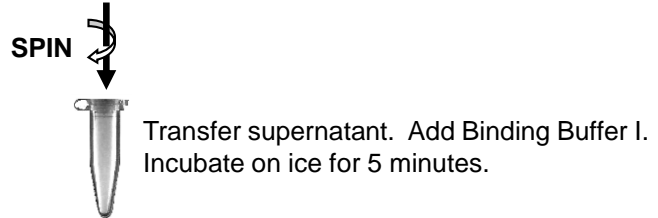
Procedure

Notes prior to use:

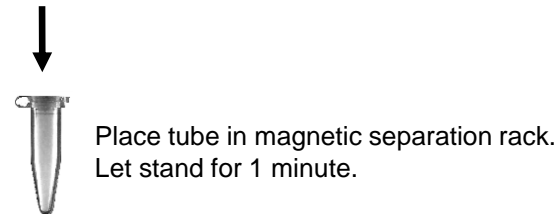
- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- **Always** vortex the **Magnetic Bead Suspension** before use.

Flow Chart
Procedure for Purifying Soil DNA using
Norgen's Soil DNA Isolation Kit (Magnetic Bead System)

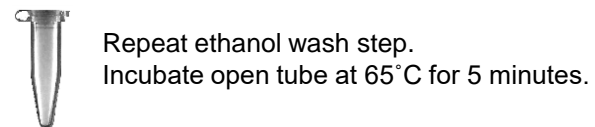
Add soil sample and Lysis Buffer G to Bead Tube, vortex. Add Lysis Additive A, vortex.



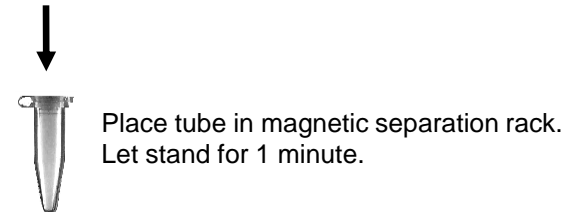
Discard supernatant. Add Buffer SK,
mix and incubate for 1 minute.



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



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



Carefully transfer supernatant
to Elution Tube.

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 $14000 \times g$ (~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 $14000 \times g$ (~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.
- 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 $14000 \times g$ (~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 2 mL DNAase-free microcentrifuge tube (not provided).
- j. Add 400 μL **Binding Buffer B** and 425 μL **96-100% ethanol** (provided by the user). Mix by vortexing.
- k. Add 40 μL of **Magnetic Bead Suspension** (vortex prior to use) to the lysate collected above. Mix by vortexing.
- l. Incubate at room temperature for 5 minutes. Occasionally invert the tube.
- m. Proceed to Section 2: Soil DNA isolation

2. Soil DNA Isolation

- a. Assemble a magnetic separation rack and place the sample tube in the magnetic rack. Allow to sit for 1 minute.
- b. Aspirate and discard supernatant without touching the magnetic beads.
- c. Remove the sample tube from the magnetic rack and gently add 500 μL of **Buffer SK**. Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute.
- d. Place the sample tube on the magnetic rack and allow to sit for 1 minute.
- e. Aspirate and discard supernatant without touching the magnetic beads.
- f. Remove the sample tube from the magnetic rack and gently add 500 μL of freshly prepared **70% ethanol**. Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute.
- g. Place the sample tube on the magnetic rack 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 tube as possible by pipetting.

- j. Incubate the open tube at 65°C for 5 minutes to dry the Magnetic beads.
- k. Add 75 μL of **Elution Buffer B**. Mix by vortexing and incubate at 65°C for 10 minute.
- l. Briefly vortex and place sample tube on the magnetic rack and allow to sit for 1 minute.

- m. Carefully transfer the elution to a fresh 1.7 mL elution tube (provided) without touching the magnetic beads. The purified DNA sample may be stored at 4°C for a few days. 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 accidentally 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 tube. Mix well, and place the tube back onto the magnetic separation rack 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 65°C 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 65°C 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 #
Stool Nucleic Acid Collection and Transport Tubes	45630, 45660
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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