

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, reproducible and 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 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 which could be extracted either manually or by using automation platform IsoPure™ and is fully compatible with downstream PCR applications, as all humic acid substances and PCR inhibitors are removed during the isolation. The protocol can also be easily modified by making minor changes to be able to run on other Magnetic Bead-based automation platforms such as KingFisher™ Flex 96 and Hamilton MagEx STAR platforms.

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 L and Lysis Additive A in the provided Bead B 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 is then repeated using the provided OSR (Organic Substance Removal) Solution for removal of organic substances. The lysate is then spun in order to remove any debris, and the lysate is then transferred into a well of the 96-well plate. Magnetic Beads B, Binding Buffer B 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 Solution WN and 80% 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 g of Soil*	1 – 5 µg
Average Purity (OD260/280)	1.7 – 1.9
Time to Complete 96 Purifications (automated)	30 minutes (hands-on time)
Time to Complete 96 Purifications (manual)	90 minutes (hands-on time)

* 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.

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 Safety Data Sheets (SDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

OSR Solution, Binding Buffer B and **Solution WN** 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.

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
- Isolate sequencing-quality total DNA from a variety of microorganisms including bacteria, fungi and algae
- Magnetic Bead protocol can easily be adapted on a variety of automation systems (IsoPure™, KingFisher, Hamilton Star/Vantage, and Tecan)
- Purified DNA is suitable for a variety of downstream applications, including qPCR or NGS sequencing

Kit Components

Component	Product #62800 (192 samples)
Lysis Buffer L	1 x 105 mL 1 x 60 mL
Lysis Additive A	25 mL
Binding Buffer I	25 mL
OSR Solution	12 mL
Binding Buffer B	85 mL
Magnetic Beads B	2 x 2.2 mL
Solution WN	4 x 55 mL
Elution Buffer B	30 mL
Bead B Tubes	8 x 25
96-Well Plate	2
96-Well Elution Plate	2
Adhesive Tape	2
Product Insert	1

Customer-Supplied Reagents and Equipment

- Magnetic Bead 96-Well Separation Plate (For manual Isolation)
- Adhesive Tape (For manual Isolation) – We recommend Norgen Cat. 28394
- IsoPure™ 96 or IsoPure™ Mini Purification System (For automated Isolation)
- Multi-channel micropipettors
- Microcentrifuge tube
- 80% ethanol (prepare fresh)
- 96 – 100% ethanol
- Temperature adjustable (37°C, 65°C) incubator(s)
- Orbital Shaker
- RNase A (optional)
- Vortex Mixer
- Ice

Procedure

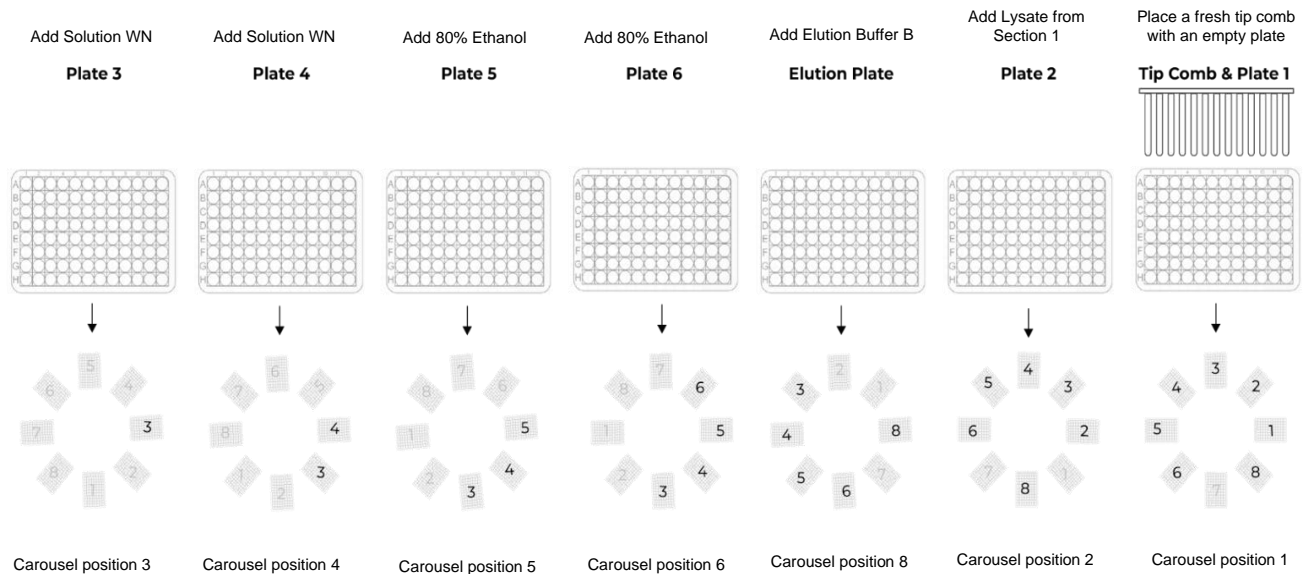
Notes prior to use:

- The procedure provides information and steps to follow to extract DNA using Automated as well as Manual method.
- **Section 1** describes the procedure to prepare lysate while **Section 2** describes automated and manual purification of DNA from the prepared lysate.
- 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 Beads B** before use.

- Preheat the incubator(s) according to the temperatures required (37°C or 65°C). For automation procedures, the automation platform that is being used may have a heat block to provide appropriate temperatures for incubation and thus an incubator might not be required. IsoPure™ instruments come with heat blocks and the scripts provided includes the incubation at required temperature.
- Prepare a working concentration of the **Solution WN** by adding 73 mL of 96 - 100 % ethanol (not provided) to the supplied bottle containing the concentrated **Solution WN**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.

Flow Chart

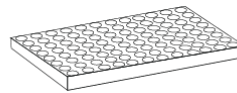
Procedure for using Norgen's Soil DNA Isolation 96-Well Kit (Magnetic Bead System) – IsoPure™ 96



Run Instrument



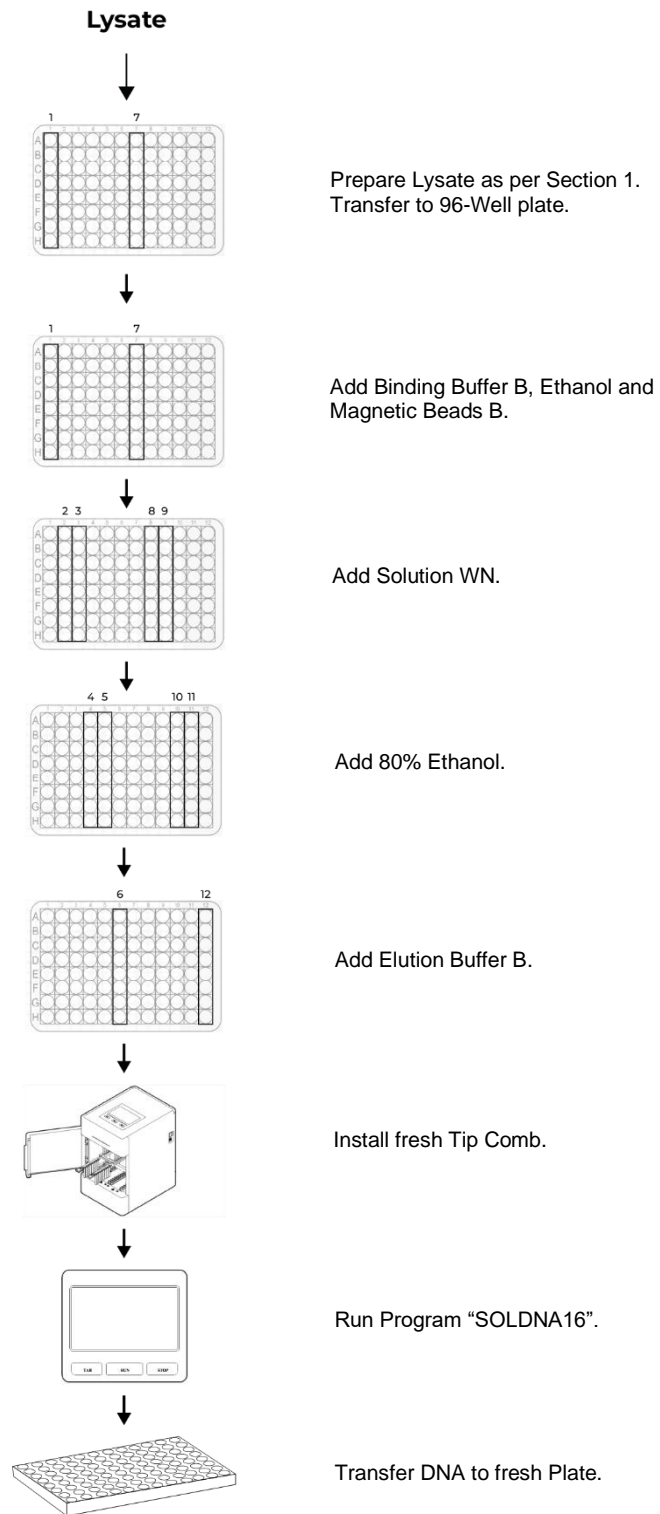
Run the Program
"SOLDNA96"



Run Complete. Take Elution
plate and store DNA.

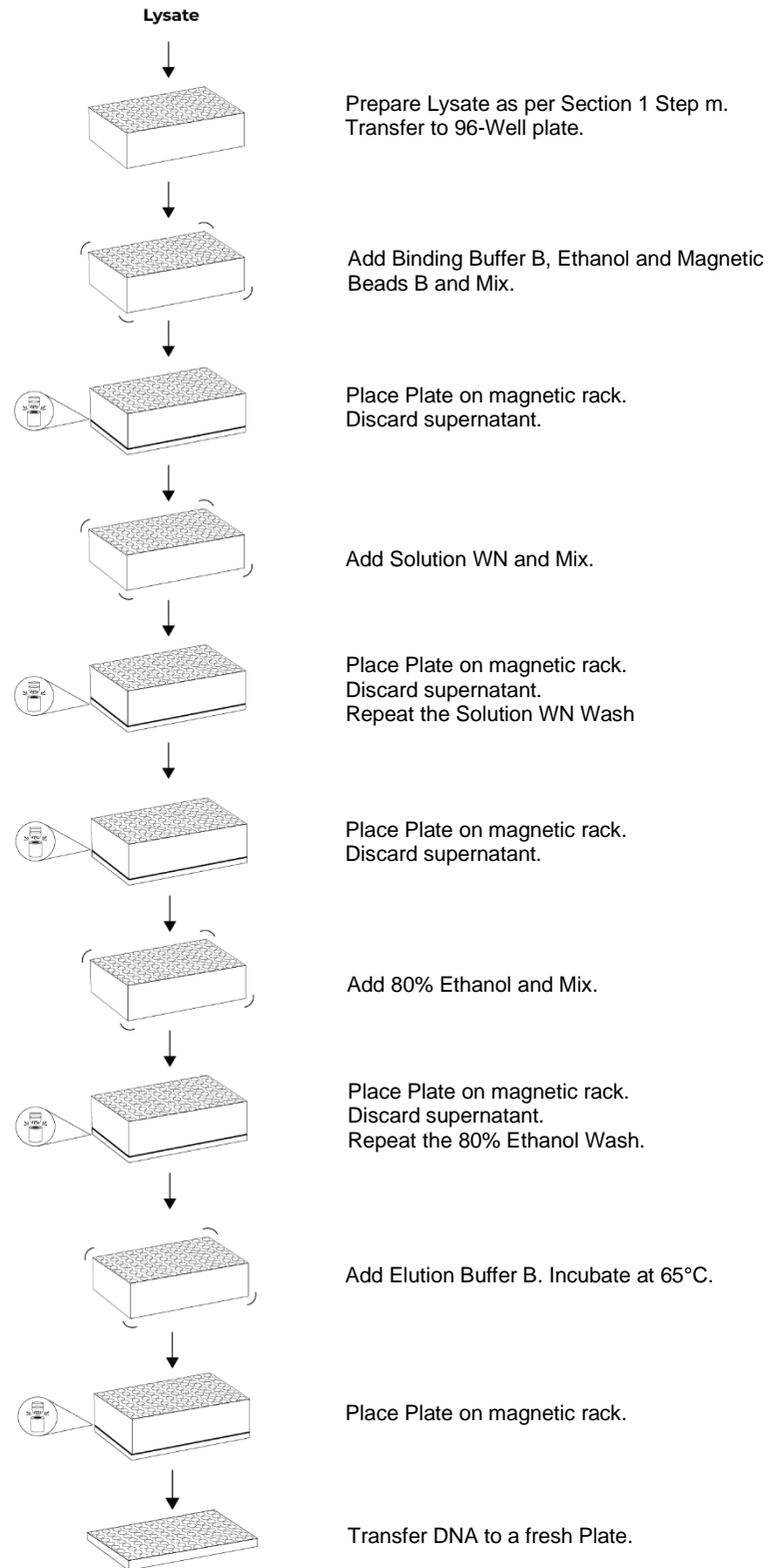
Flow Chart

Procedure for using Norgen's Soil DNA Isolation 96-Well Kit (Magnetic Bead System) – IsoPure™ Mini



Flow Chart

Procedure for Norgen's Soil Isolation 96-Well Kit (Magnetic Bead System) - Manual Method



Soil DNA Isolation using Automation and Manual Method

This section outlines step-by-step instructions for soil DNA extraction using both automated and manual methods. The automation method provides guidance for using IsoPure™ instruments – IsoPure™ 96 (Cat. AP1096) and IsoPure™ Mini (Cat. AP1016), however this protocol can also be easily adapted for other magnetic bead-based automation platforms, such as KingFisher™ Flex 96 and Hamilton MagEx STAR platforms.

Section 1 details the preparation of lysates from soil sample, applicable to both automated and manual DNA extraction methods.

Section 2 is divided into multiple subsections, offering detailed instructions for extracting DNA from the lysate prepared in **Section 1**, using either automated systems or manual procedures.

To prepare for Soil DNA extraction using automated systems, please follow the steps outlined below.

Set-up the IsoPure™ Plates

Notes Prior to Use

- Only use plates that are compatible with the instrument that is being used. Use 96 deep well plates for IsoPure 96 (Cat. AP1096-DWP), 96 well elution plates for IsoPure™ (Cat. AP1096-ELP) and Tip Comb for IsoPure 96 (Cat. AP1096-TC) while working with IsoPure™ 96 Instruments. Use 96 deep well plates for IsoPure 96 (Cat. AP1096-DWP) and 8-place magnetic tip comb for IsoPure Mini (Cat. AP1016-MC) while working with IsoPure™ Mini Instruments.
- The numbering of plates is based on where it should be placed on the carousel in the instrument. Use the carousel rotation keys on the instrument or select the carousel position on the screen to bring it to the right position as mentioned in the flow chart.
- IsoPure™ 96 uses multiple plates for DNA extraction while IsoPure™ Mini uses single plate for DNA extraction.
- For IsoPure™ 96 most of the lysate preparation is performed in plate 2 while other plates are used for performing washing and elution steps.
- For IsoPure™ Mini the lysate preparation is performed in column 1 and/or 7 while washing and elution steps could be performed in other columns of the same plate.
- For IsoPure™ 96, DNA elution step is always performed in plate 8.
- Prepare the plates and label them based on the sample type as mentioned in the tables below.

Table 1. Set-up for Soil DNA Extraction

Prepare plates as per the volumes mentioned in the table below. Detailed description of step-wise process can be found in **Section 1 and 2**.

Component	IsoPure™ Mini	IsoPure™ 96	Location on Carousel	Amount
Tip Comb	In the instrument	Plate 1	1	Empty Plate
Lysate	Column 1 / Column 7	Plate 2	2	Variable
Solution WN	Column 2 / Column 8 and Column 3 / Column 9	Plate 3 & Plate 4	3 & 4	1 mL
80% Ethanol	Column 4 / Column 10 and Column 5 / Column 11	Plate 5 & Plate 6	5 & 6	1 mL
Elution Buffer B	Column 6 / Column 12	Elution Plate	8	100 µL

Section 1. Soil Sample Collection and Lysate Preparation

- a. Add up to 250 mg of soil sample to a provided Bead B Tube and add 750 μL of **Lysis Buffer L**. Vortex briefly to mix soil and lysis buffer.

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 $20,000 \times g$ (~14,000 RPM). Remove the water carefully using a pipette and resuspend the soil pellet in 750 μL of **Lysis Buffer L**. Transfer the soil and lysis buffer mixture to a Bead B Tube using a pipette.

- b. Add 100 μL of **Lysis Additive A** and vortex briefly.
- c. Secure bead tube to a vortex mixer and vortex at maximum speed for 10 minutes.
- d. Centrifuge the tube for 2 minutes at $20,000 \times g$ (~14,000 RPM).
- e. Transfer up to 450 μL of supernatant to a fresh 96-well plate (not provided) or 1.7 mL DNase-free microcentrifuge tube (not provided).
- f. Add 100 μL of **Binding Buffer I** to each sample.
- g. Seal the plate with an Adhesive Tape (not provided) and mix the 96-well plate or tube by inverting a few times and incubate for 5 minutes on ice or at -20°C .
- h. Spin the 96-well plate for 2 minutes at $3220 \times g$ (~4,000 RPM) to pellet any protein and soil particles (If using tubes, spin them at $20,000 \times g$ (~14,000 RPM) for 2 minutes).
- i. Transfer up to 450 μL of supernatant to a fresh 96-well plate (not provided) or 1.7 mL DNase-free microcentrifuge tube (not provided) without any contact with the pellet.
- j. Add 50 μL of **OSR Solution** to each sample.
- k. Seal the plate with an Adhesive Tape (not provided) and mix the plate on an orbital shaker at 1000 rpm for 2 minutes, followed by incubation on ice or at -20°C for 5 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate on ice or at -20°C for 5 minutes. If using a tube, mix by brief vortexing, and incubate on ice or at -20°C for 5 minutes.
- l. Spin the 96-well plate for 2 minutes at $3220 \times g$ (~4,000 RPM) to pellet any protein and soil particles (If using tubes, spin them at $20,000 \times g$ (~14,000 RPM) for 2 minutes).
- m. Transfer up to 340 μL of supernatant (avoid contacting the pellet with the pipette tip) into **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or **96-Well Plate** (Manual extraction).
- n. Prepare a mixture of 300 μL **Binding Buffer B**, 320 μL **96-100% ethanol** (not provided) and 20 μL of **Magnetic Beads B** (vortex prior to use) to the lysate collected above. We recommend a 10% overage while making mixture for multiple samples, to compensate for pipetting errors. Make sure to vortex the bulk mix before adding it to the lysate. It is recommended to prepare the bulk fresh every time a new DNA isolation is performed.

Optional RNase A Treatment:

If RNA-free genomic DNA is required, add the equivalent of 10 KUnitz of **RNase A** (not provided), make sure not to exceed 20 μL , to the lysate with **Binding Buffer B**, **96-100% ethanol** and **Magnetic Beads B** mixture to **Plate 2** (IsoPure™ 96), **Column 1 and/or Column 7** (IsoPure™ Mini) or **96-Well Plate** (Manual extraction) during this step. If RNase treatment is performed, make sure to run program **SOLDNA96R** (IsoPure™ 96) program instead of **SOLDNA96** and **SOLDNA16R** (IsoPure™ 16) program instead of **SOLDNA16** to include an incubation step of 37°C for 15 minutes.

- o. Proceed to Section 2: **DNA Isolation from Soil Lysate**.

Section 2: DNA Isolation from Soil Lysate

2.1 DNA Isolation using IsoPure™ 96 System

- a. Setup plates according to **Table 1**. Add 1 mL **Solution WN** (added with ethanol) to **plate 3 & 4** and keep it at **position 3 & 4** respectively.
- b. Add 1 mL **80% ethanol** to **plate 5 & 6** and keep it at **position 5 & 6** respectively.
- c. Add 100 μL of **Elution Buffer B** to the **Elution plate** and place it at **position 8** on the carousel.

- d. Place sample plate (**Plate 2**) in the machine at **position 2**.
- e. Place a clean tip comb in an empty plate (**Plate 1**) and keep it at **position 1**.
- f. After setting up, run the program **SOLDNA96** or **SOLDNA96R**.
- g. The instrument will prompt after the procedure is complete. Remove the elution plate from the instrument and the DNA is now ready for further downstream processing.
- h. The purified DNA sample may be stored at 4°C for a few days. The provided Adhesive Tape can be used to seal the **Elution Plate** for storage of the DNA. It is recommended that samples be placed at –20°C for long-term storage.

2.2 DNA Isolation using IsoPure™ Mini System

- a) Add 1 mL **Solution WN** (added with ethanol) to **Column 2 and/or Column 8** as well as **Column 3 and/or Column 9**.
- b) Add 1 mL **80% ethanol** to **Column 4 and/or Column 10** as well as **Column 5 and/or Column 11**.
- c) Add 100 µL of **Elution Buffer B** to **Column 6 and/or 12**.
- d) Place a clean tip comb into IsoPure™ Mini.
- e) After setting up, place the plate into the instrument and run the program **SOLDNA16** or **SOLDNA16R**.
- f) The instrument will prompt after the procedure is complete. Remove the plate from the instrument and the DNA is now ready for further downstream processing.
- g) Transfer the eluted DNA from **Column 6 and/or Column 12** to a fresh plate. The purified DNA sample may be stored at 4°C for a few days. The provided Adhesive Tape can be used to seal the plate for storage of the DNA. It is recommended that samples be placed at –20°C for long-term storage.

2.3 DNA Isolation using Manual Method

- a) Seal the plate with an Adhesive Tape (not provided) and mix the plate on an orbital shaker at 1000 rpm for 5 minutes. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 5 minutes.
- b) Place the 96-Well Plate on the magnetic plate. Allow to sit for 1 minute. Incubate for more time if the solution is not clear.
- c) Remove the Adhesive Tape. Aspirate and discard supernatant without touching the magnetic beads.
- d) Remove the 96-Well Plate from the magnetic plate and gently add 1 mL of **Solution WN (ensure ethanol was added)**.
- e) Seal the plate with an Adhesive Tape (not provided) and mix the plate on an orbital shaker at 1000 rpm for 1 minute. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 1 minute.
- f) Place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute. Incubate for more time if the solution is not clear.
- g) Aspirate and discard supernatant without touching the magnetic beads.
- h) Repeat **Steps d – g from this section** for a second wash step.
- i) Remove the 96-Well Plate from the magnetic plate and gently add 1 mL of freshly prepared **80% ethanol**.
- j) Seal the plate with an Adhesive Tape (not provided) and mix the plate on an orbital shaker at 1000 rpm for 1 minute. If orbital shaker is not available, mix the lysate 10 times using a pipette and incubate at room temperature for 1 minute.
- k) Place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute. Incubate for more time if the solution is not clear.
- l) Aspirate and discard supernatant without touching the magnetic beads.
- m) Repeat **Steps i – l from this section** for a second wash step.

Note: Remove as much of the **80% ethanol** in the sample plate as possible by pipetting.

- a) Incubate the 96-Well plate at room temperature for 5 to 10 minutes to air-dry the magnetic beads. Make sure not to over-dry the magnetic beads as this might affect the DNA yield.
- n) Remove the sample plate from the magnetic plate and add 100 µL of **Elution Buffer B**. Mix by gently pipetting and incubate at 65°C for 10 minutes.

- o) Briefly mix by pipetting and place the 96-Well Plate on the magnetic plate and allow to sit for 1 minute. Incubate for more time if the solution is not clear.
- p) Carefully transfer the elution to a **96-Well Elution Plate** (provided) without touching the magnetic beads.
- q) The purified DNA sample may be stored at 4°C for a few days. The provided Adhesive Tape can be used to seal the **96-Well Elution Plate** for 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 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 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 65°C may result in increased yields.
	Amount of magnetic beads added was not sufficient	Ensure that the magnetic bead B 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 contains 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 Solution WN	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 Solution WN. 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 80% 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 Plus Kit (50 prep)	64000
Soil Nucleic Acid Isolation Kit	45600
Adhesive Tape – 100 Units	28394

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