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Instruction Manual

SPINeasy DNA Kit for Soil

Spin Columns for Quick Isolation of Genomic DNA from Soil

Cat. No.: 116530050 (50 Preps)

Storage: 15-30 ˚C

Table of Contents

1. Introduction

SPINeasy DNA Kit for Soil is a high–performance soil gDNA extraction kit based on spin–column technology. It enables quick isolation of gDNA from soil in less than 30 min. Briefly, soil samples are placed into Lysing Matrix E tubes and used with FastPrep® Instruments from MP Biomedicals to effectively lyse various cells including bacteria, fungi, viruses, protists and more. A series of specially formulated reagents were developed to remove humic substances and other contaminants. Column S1 provided in the kit has high binding capacity and selectivity for gDNA. The combined components of the kit provide an indispensable tool for extracting high yields of pure gDNA from even the most difficult environmental samples. Extracted gDNA is ready for downstream analyses such as PCR, restriction digestion and sequencing. Visit www.mpbio.com to explore additional products to support your research.

2. Kit Components and User Supplied Materials

2.1 Kit Components

Components	Package	Cat. No.
Lysing Matrix E	50 ea	116914050
Lysis Buffer S1	60 mL	116530051
Lysis Buffer S2	8 mL	116530052
RNase A Solution	550 µL	116530053
Inhibitor Removal S	15 mL	116530054
Binding Buffer S	15 mL	116530055
Wash Buffer S	6 mL	116530056
DES Buffer	10 mL	116530057
Column S1	50 ea	116530058
2.0 mL Collection Tubes	50 ea	116530059
1.5 mL Collection Tubes	50 ea	116530060
Instruction Manual	1 ea	–
Quick–Start Protocol	1 ea	–
MSDS & CoA	Available www.mpbio.com	

2.2 User Supplied Materials

- FastPrep® Instrument – FastPrep–24™ 5G (Cat. No.116005500) or Vortex with an adapter
- Microcentrifuge capable of at least 14,000 x g
- Water bath or heat block
- Absolute ethanol (85 mL)
- 2.0 mL Microcentrifuge tubes (100 pcs)

3. Storage and Stability

All SPINeasy DNA Kit for Soil components are guaranteed for at least 24 months from the date of manufacture when stored at room temperature (15–30 ℃).

4. Notes Before Starting

Please tick as appropriate.

☐ Expect precipitation in Lysis Buffer S1, warm the solution to 55 ℃ will dissolve the precipitate.

☐ Add 35 mL absolute ethanol to Binding Buffer S and mark on the bottle.

☐ Add 50 mL absolute ethanol to Wash Buffer S and mark on the bottle.

☐ Vortex the sample at full speed for 10 min if a FastPrep® Instrument is unavailable. Secure samples on the vortex through an adapter to ensure homogenization.

5. Safety Precautions

Lysis Buffer S2 contains components that may cause irritation when in contact with human tissue. Wear personal protective equipment (gloves, lab coat and eye protection) to prevent contact with the skin or mucous membranes. Consult the Material Safety Data Sheet at www.mpbio.com for additional details.

6. Protocol

A Quick-Start Protocol is provided in the kit for quick reference throughout the extraction process.

1. Add 100–500 mg soil sample to **Lysing Matrix E** tube.
   Note: SPINeasy DNA Kit for Soil can extract gDNA from a wide range of soil samples. The amount of starting material is dependent on the biomass level in the selected soil type. Soil of high biomass, such as flowerbed soil and topsoil, are only required at 100 mg for the extraction process.
2. Add 980 µL **Lysis Buffer S1**, 120 µL **Lysis Buffer S2** and 10 µL **RNase A Solution** to the sample; vortex to mix.
3. Homogenize in a FastPrep® Instrument for 20 seconds at speed setting of 6.0 m/s.
   Note: Vortex the sample at full speed for 10 min if a FastPrep® Instrument is unavailable. Secure samples on the vortex through an adapter to ensure homogenization.
4. Centrifuge at 14,000 x g for 5 min.
   Note: Centrifuge at the maximum speed for all steps if 14,000 x g is not feasible.
5. Carefully transfer the supernatant to a clean 2.0 mL microcentrifuge tube (self-provided).
6. Add 250 µL **Inhibitor Removal S** to the transferred supernatant and shake 10 times to mix.
7. Centrifuge at 14,000 x g for 10 min.
8. Transfer 900 µL supernatant to a clean 2.0 mL microcentrifuge tube (self-provided).
9. Add 900 µL of **Binding Solution S** to the transferred supernatant.
10. Transfer 800 µL of the mixture to **Column S1** placed on top of a **2.0 mL Collection Tube** (provided).
11. Centrifuge at 14,000 x g for 1 min. Empty collection tube and reuse. Repeat the process once and discard the remaining mixture.
12. Add 500 µL **Wash Buffer S** to the column. Centrifuge at 14,000 x g for 1 min. Empty collection tube and reuse. Repeat the washing step.
13. Without addition of any liquid, centrifuge the empty column at 14,000 x g for 2 min to dry it.
14. Discard collection tube and place the column into a **1.5 mL Collection Tube** (provided).
15. Air dry the column for 5 min at room temperature.
16. Heat **DES Buffer** to 55 ℃ using a water bath while waiting.
17. Add 100 µL of pre-heated **DES Buffer** to the center of column.
18. Centrifuge at 14,000 x g for 1 min to elute DNA.
19. Eluted DNA is now ready for downstream applications. Store at −20 ℃ for extended periods or 4 ℃ until use.

7. Data

SPINeasy DNA Kit for Soil has been extensively tested for its performance. The following table displays gDNA yields obtained from various soil samples using the kit. Results demonstrate high yields and purity of extracted gDNA, ready-to-use for PCR amplification.

Table 1: Quality and quantity of gDNA extracted from various soil samples using SPINeasy DNA Kit for Soil.

Sample	Extraction Results		
	Yield (ng/mg sample)	A260/280	A260/230
Organic soil	47.78 ± 0.21	1.79 ± 0.01	1.10 ± 0.02
Paddy soil	16.03 ± 0.06	1.94 ± 0.01	1.70 ± 0.04
Flowerbed soil	14.88 ± 0.65	1.84 ± 0.01	1.20 ± 0.04
Saline soil	7.55 ± 0.65	1.89 ± 0.01	1.40 ± 0.00
Desert soil	1.64 ± 0.19	1.85 ± 0.06	1.02 ± 0.00

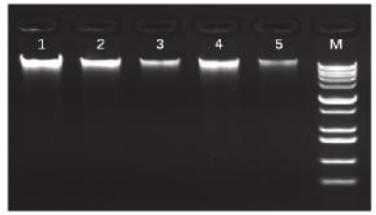

Figure 1:
gDNA extracted from different soil samples using SPINeasy DNA Kit for Soil, analyzed using 1% agarose gel electrophoresed at 70 V for 30 min.
M: 1kb plus DNA ladder;
Lane 1: 100mg Organic soil (loading 4 μ L);
Lane 2: 100mg Paddy soil (loading 8 μ L);
Lane 3: 100mg Flowerbed soil (loading 8 μ L);
Lane 4: 250mg Saline soil (loading 8 μ L);
Lane 5: 250mg Desert soil (loading 8 μ L).

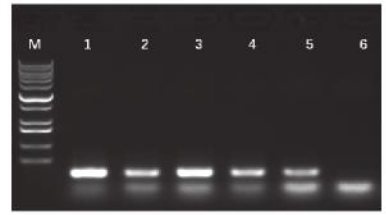

Figure 2:
PCR amplification of 16S rRNA gene from different soil samples using SPINeasy DNA Kit for Soil, analyzed using 1 % agarose gel electrophoresed at 70 V for 30 min.
M: 1kb plus DNA ladder;
Lane 1: Organic soil;
Lane 2: Paddy soil;
Lane 3: Flowerbed soil;
Lane 4: Saline soil;
Lane 5: Desert soil;
Lane 6: Negative control.

8. Troubleshooting

8.1 Sample Handling

1. SPINeasy DNA Kit for Soil is not only suitable for soil, but also for a wide range of environmental samples, including wastewater, stool, rhizosphere, gypsum, garbage, sludge, sediment, etc.

2. Wet samples: If the soil is wet, remove the water using the following protocol. First, transfer the components of Lysing Matrix E to another sterile holding tube. Then, place wet soil in the empty matrix tube and centrifuge at 10,000 x g for 30 seconds. Decant as much liquid as possible, replace Lysing Matrix E components and continue with the protocol.

8.2 Low DNA Yields

1. Ensure the extraction was performed as per the manual protocol.
2. Low microbiological content: (i) Increase amount of starting material; (ii) Process multiple samples using several Lysing Matrix tubes and then pool the samples.
3. Insufficient lysis: While a FastPrep® speed setting of 6.0 m/s and 20 seconds run time will be adequate for most soil types, additional processing may be necessary. Homogenization time can be extended up to 40 seconds. When lysing using a vortex method, the lysing matrix tube should be well secured on the vortex through an adapter to ensure homogenization of the sample.
4. Increase DNA capture: Instead of transferring 2 x 800 μL of DNA–Binding Buffer S mixture to the column, transfer the entire volume.
5. Poor elution: (i) Ensure the DES Buffer is heated to 55 ℃ and is loaded to the center of the column during elution; (ii) Incubate the column with added DES Buffer for 5 min at 55 ℃ prior to elution.

8.3 Low A260/280 Ratios for Purified DNA

1. Proteins not removed efficiently: Inhibitor Removal S must be efficiently mixed in the lysate. Invert tube by hand at least 10 times or mix by pipet pumping. Incubating the sample on ice/ keeping it in the fridge for 5 min can further precipitate proteins from difficult samples.
2. Contaminants not removed efficiently: Washing should be carried out twice using Wash Buffer S.

8.4 High A260/280 Ratios for Purified DNA

Possible RNA contamination, which can be confirmed via gel electrophoresis analysis. Incubate sample with RNase A Solution for 5 min after the lysis step before spinning down the debris.

Alternatively, you can add 20 μL or more of RNase A Solution to the lysis system.

8.5 Low A260/230 Ratios for Purified DNA

1. Proteins not removed efficiently: Refer to 8.3.1.
2. Contaminants not removed efficiently: Refer to 8.3.2.
3. Residual ethanol in eluted DNA: (i) Increase centrifugation speed or time to dry spin the column, (ii) Increase the air-drying time of Column S1 or (iii) Incubate the column in a 55 ℃ oven to speed up the drying process.

8.6 Fragmented DNA

Optimize lysis conditions: High powered bead beating cell disrupters can shear DNA if process settings are too long or powerful. While a FastPrep® speed setting of 6.0 m/s and 20 seconds run time will be adequate for most soil types, it is possible that lowering the speed and/or duration settings will result in higher molecular weight DNA.

8.7 DNA Does Not Amplify

1. Quantify DNA yield using a spectrophotometer. High concentrations of DNA will inhibit PCR reactions.
2. Dilute DNA template: Inhibitors in the eluted DNA can inhibit PCR reactions. Dilution of template DNA can reduce such inhibition. This should not be necessary with DNA isolated with the SPINeasy DNA Kit for Soil, but is still an option.
3. Verify PCR optimization conditions: Changing reaction conditions or primer selection may be necessary.

   Non-specific bands: Check possibility that target DNA is in low abundance in the eluate. It is possible that some species of interest, particularly parasitic cysts and oocytes, may need additional processing or even more aggressive lysing matrix (such as Lysing Matrix A, Cat. No.116910050) to disrupt the thick protein cell wall.

9. Product Use Limitations & Warranty

The products presented in this instruction manual are for research or manufacturing use only. They are not to be used as drugs or medical devices in order to diagnose, cure, mitigate, treat or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery. Buyer's exclusive remedy and the sole liability of MP Biomedicals hereunder shall be limited to, at our discretion, no replacement or compensation, product credits, refund of the purchase price of, or the replacement of materials that do not meet our specification. By acceptance of the product, Buyer indemnifies and holds MP Biomedicals harmless against, and assumes all liability for, the consequence of its use or misuse by the Buyer, its employees or others, including, but not limited to, the cost of handling. Said refund or replacement is conditioned on Buyer notifying within thirty (30) days of receipt of product. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s). FastDNA®, FastRNA®, FastPrep®, QBiogene®, and BIO 101® Systems are registered trademarks of MP Biomedicals, LLC.