

Contents

Introduction	2
Overview	2
Storage and Stability	2
Kit Contents	3
Before Starting	3
Soil DNA Isolation Protocol	4
Troubleshooting	7

Introduction

E.Z.N.A.™ Soil DNA Kits allow rapid and reliable isolation of high-quality total DNA from various soil samples. Up to 1 gram of soil samples can be processed in less than 60 minutes. The system combines the reversible nucleic acid-binding properties of HiBind™ matrix with the speed and versatility of spin column technology to eliminate PCR inhibiting compounds such as humic acid from soil samples. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

Overview

Soil samples typically contain many compounds that can inhibit downstream enzymatic reactions. E.Z.N.A.™ Soil DNA Kit contains SL1 Buffer, a unique reagent to remove those compounds during early stage of DNA purification procedure. In addition, this also contains a SL2 Buffer, which is specially designed to remove inhibitory substances from soil sample.

If using the E.Z.N.A.™ Soil DNA Kit for the first time, please read this booklet to become familiar with the procedure. Soil sample is homogenized and then treated in a specially formulated buffer containing detergent. Humic acid, proteins, polysaccharides, and other contaminants are subsequently precipitated after a heat-frozen step. Contaminants are further removed by isopropanol precipitation of DNA. Binding conditions are then adjusted and the sample is applied to an HiBind™ DNA spin-column. Two rapid wash steps remove trace contaminants and pure DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the E.Z.N.A.™ Soil DNA Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C. During shipment, or storage in cool ambient conditions, precipitates may form in some buffers. It is possible to dissolve such deposits by incubation the solution at 65°C.

Kit Contents

Product Number	D5625-00	D5625-01	D5625-02
HiBind™ DNA Columns	5	50	200
2 ml Collection Tubes	15	150	600
Glass Beads	2.7 g	55 g	210 g
SL1	5 ml	50 ml	180 ml
SL2	2.5 ml	10 ml	40 ml
SL3	1.0 ml	10 ml	40 ml
HB Buffer	3 ml	30 ml	110 ml
Proteinase K	3 mg	30mg	4 x 30 mg
DNA Elution Buffer	1.5 ml	15 ml	50 ml
Wash Buffer Concentrate	12 ml	40 ml	160 ml
Instruction Booklet	1	1	1

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.™ Soil DNA Miniprep Kit procedure.
- Prepare an SL 1 stock solution by add 10µl 2-mercaptoethanol per 1 ml SL1 before use. Each sample will require 600 µl of this solution.
- Dilute DNA Wash Buffer Concentrate with ethanol as follows and store at room temperature.

D5625-00 Add 18 ml absolute (96%-100%) ethanol.

D5625-01 Add 60 ml absolute (96%-100%) ethanol to each bottle.

D5625-02 Add 240 ml absolute (96%-100%) ethanol to each bottle.

- Prepare proteinase K stock solution as following:

D5625-00 Add 125µl Elution Buffer to the vial

D5625-01 Add 1.25 ml Elution Buffer to the vial

D5625-02 Add 1.25 ml Elution Buffer to the vial

Soil DNA Isolation Protocol

Materials to be provided by user:

- Microcentrifuge capable of at least 10,000 x g
- Nuclease-free 1.5 ml or 2 ml microfuge tubes
- Water bath equilibrated to 65°C
- Equilibrate sterile dH₂O water or 10 mM Tris pH 9.0 at 65°C.
- 2-mercaptoethanol
- Isopropyl alcohol (isopropanol)
- Absolute (96%-100%) ethanol
- RNase A stock solution at 20 mg/ml

1. **Weight 500 mg glass beads in a 2 ml centrifuge tube, add 0.2-1 g soil sample. Add 600µl SL1 /2-mercaptoethanol solution.** Vortex at maximum speed for 3 minute or until the sample is thoroughly homogenized.
2. **Incubate at 70°C for 5 min. Mix sample twice during incubation by vortexing the tube.**
3. **Incubate the sample on ice for 5 minutes.**
4. **Vortex the sample for 30 seconds and centrifuge the sample at full speed in a microcentrifuge for 1 minutes to pellet the soil particles. Carefully aspirate 400µl supernatant to a new 2 ml microfuge tube making sure not to disturb the pellet or transfer any debris.**
5. **Add 400µl of SL2 Buffer followed by 25µl of Proteinase K. Mix the sample by vortexing.**
6. **Incubate at 70°C for 10 minutes.** Centrifuge briefly to collect any drop from the tube lid.
7. To the lysate, add equal volume (800µl) of chloroform:isoamyl alcohol (24:1) and vortex to mix. Centrifuge 10,000 x g for 2 min at room temperature. Carefully transfer the **upper** aqueous phase to a clean 1.5 ml microfuge tube. Avoid the milky interface containing contaminants and inhibitors.

8. Add 0.7 x vol isopropanol and mix to precipitate DNA. Incubate at room temperature 2 min and centrifuge 10,000 x g for 10 min. Carefully discard as much supernatant as possible without disturbing pellet. Air dry 2 min at room temperature.
9. Resuspend DNA pellet in 300 µl sterile deionized water. This may require incubation at 60°C-70°C for 10 minutes or more. Add 20 µl RNase A (20 mg/ml) and mix.
10. Adjust binding conditions by adding 150 µl Buffer SL3 followed by 300 µl absolute ethanol and vortex to mix. Apply entire mixture, including any precipitation that may have formed, to an HiBind® DNA column assembled in a 2 ml collecting tube (supplied). Centrifuge at $\geq 8,000$ x g for 1 min at room temperature. Discard flow-through liquid and collecting tube.
11. Place column into another a clean 2 ml collecting tube (supplied) and wash by adding 500 µl HB Buffer. Centrifuge at 10,000 x g for 30 seconds. Discard the flow-through and collection tube.
12. Place column into new 2 ml collecting tube (supplied) and wash by adding 750 µl DNA Wash Buffer diluted with absolute ethanol. Centrifuge 10,000 x g 1 min as above. Discard flow-through liquid and reuse collecting tube in next step.
13. Repeat step 12 with a second 750 µl DNA Wash Buffer. Discard liquid and re-insert the column to the empty collecting tube, centrifuge the column at full speed for 1 min at room temperature. This step is critical in removing traces of ethanol that will interfere with downstream applications (such as agarose gel electrophoresis of high molecular weight DNA).
14. Place column into a clean 1.5 ml microfuge tube (not supplied). To elute DNA add 50 µl-100 µl of DNA Elution Buffer (or 10 mM Tris buffer, pH 9.0) preheated to 60°C-70°C directly onto the HiBind® matrix. Allow to soak for 2 min at room temperature. Centrifuge at full speed for 1 min to collect DNA.
15. Repeat elution step with a second aliquot of DNA Elution Buffer. Typically a total of 5-15 µg DNA with absorbance ratio (A_{260}/A_{280}) of 1.7-1.9 can be obtained from 1 gram soil sample. Yields vary depending on source and quantity of starting material used.
TIP: To increase DNA concentration add buffer and incubate the column at 60°C-70°C for 5 min before elution.

Troubleshooting

Problem	Cause	Suggestions
A260/280 ratio is low	inefficient elimination of inhibitory compounds	Repeat the DNA isolation with a new sample, be sure to mix the sample with SL2 Buffer thoroughly.
	DNA pellet not completely dissolved before applying sample to column.	Ensure that DNA is dissolved in water before adding Buffer SL3 and ethanol. This may need repeated incubation at 65°C and vortexing.
	No ethanol added to the lysate before loading to the column	Repeat the DNA isolation with a new sample.
	DNA wash Buffer prepared with lower percentage ethanol	prepare DNA Wash Buffer with 96-100% ethanol

A260/280 ratio is high	RNA contamination	Be sure to treat the sample with RNase A in step 9.
Low DNA yield or no DNA eluted	Sample stored incorrectly	Sample should be store at 4°C or -20°C
	Poor homogenization of sample.	Repeat the DNA isolation with a new sample, be sure to mix the sample with PBS/SL1 thoroughly.
	No ethanol added to the lysate before loading to the column	Repeat the DNA isolation with a new sample
	DNA washed off.	Dilute Wash Buffer Concentrate by adding appropriate volume of absolute ethanol prior to use (page 3).
Problems in downstream applications	BSA no added to PCR mixture	Add BSA to a final concentration of 0.1 µg/ml to the PCR mixture.
	Too much DNA inhibit PCR reaction	Diluted the DNA elute used in the downstream application if possible.
	No-specific bands in downstream PCR	Use hot-start Taq polymerase mixture
	inhibitory substance in the eluted DNA.	Check the A260/280 ration Diluted the elute to 1:50 if necessary
	Ethanol residue in the elute	Be sure to completely dry the column before elution

Little or no supernatant after initial centrifuge step	Insufficient centrifugal force	Check the centrifugal force and increase the centrifugal time if necessary
sample can not pass through the column	Clogging column	Check the centrifugal force and increase the time of centrifugation