	inhibitor substance in the elute DNA	Check the A260/230 ration. Dilute the elute to 1:50 if necessary
	Ethanol residue in the elute	Be suer 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

If the above suggestions fail to resolve any problems you are having with the E.Z.N.A.® Soil DNA Kit, please feel free to fax our technical specialists at:
US customers: 800-832-8896 or 770-931-8400
All other customers: (770) 931-0230
Or direct your questions via E-mail to info@omegabiotek.com.

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Revised Feb 2009

B. Purification of Inhibitor-Free Soil DNA Obtained by other Methods

Soil DNA isolated by other methods can be further purified using E.Z.N.A.™ Soil DNA Kit.

1. **Dissolve the DNA pellet with 200 µl of Elution Buffer or Buffer TE.**

   Important: DNA pellet should be dissolved in Elution Buffer or Buffer TE(pH8.0).

2. **Add 50 µl inhibitor Removal Resin to the sample and vortex to mix well.**

   Note: Completely resuspend HTR Reagent by shaking the bottle before use.

3. Incubate at room temperature for 2 min and centrifuge at maxi speed (≥13,000 x g) for 2 min to remove Inhibitor Removal Resin.

4. **Carefully transfer the supernatant into a new tube.**

   Note: If supernatant still have dark color from soil, perform the HTR treatment again by repeating step 2-4.

5. Follow Step 12-21 on page 5.

Kit Contents

Product Number	D5625-00	D5625-01	D5625-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind™ DNA Column	5	50	200
2 ml Collection Tube	10	100	400
Glass Beads	3 g	30 g	110 g
HTR Reagent	1.2 ml	6 ml	25 ml
Buffer SLX Mlus	6 ml	60 ml	220 ml
Buffer DS	0.6 ml	6 ml	22 ml
Buffer SP2	3 ml	20 ml	60 ml
Buffer XP2	3 ml	30 ml	120 ml
Elution Buffer	1.5 ml	20 ml	80 ml
SPW Wash Buffer	2 ml	25 ml	3 x 25 ml
Instruction Booklet	1	1	1

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.™ Soil DNA Kit protocol.
- Dilute SPW Wash Buffer with absolute ethanol as follows and store at room temperature.

D5625-00	Add 8 ml (96%-100%) ethanol.
D5625-01	Add 100 ml (96%-100%) ethanol to bottle
D5625-02	Add 100 ml (96%-100%) ethanol to each bottle

- Preheat Elution Buffer at 70°C

Introduction

The E.Z.N.A.™ Soil DNA Kit allows rapid and reliable isolation of high-quality genomic 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

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 extraction steps. 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 should be stored at 22°C-25°C. Under these conditions, DNA has successfully been purified and used for PCR after 24 months of storage. 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 55°C.

Troubleshooting Guide

Problem	Cause	Suggestions
A260/230 ratio is low	inefficient elimination of inhibitory compounds	Repeat the DNA isolation with a new sample, be sure to mix the sample with HTR Reagent and mix the sample throughly.
	Salt contamination	Repeat the DNA isolation with a new sample. Make sure the column is dried before the elution. Wash the column with extra SPW Wash Buffer.
A260/280 is high	RNA contamination	Treat the sample with Rnase A in step 8.
Low DNA Yield or no DNA Yield	Sample stored incorrectly	Sample should be store at -20°C
	Poor Homogenization of Sample	Repeat the DNA isolation with a new sample, be sure to mix the sample with SLX throughly.
	Incorrect Buffer xp2 was added before loading to the column	Repeat the DNA isolation with a new sample
	DNA Wash off.	Dilute SPW Wash Buffer by adding appropriate volume of absolute ethanol prior to use.
Problems in downstream applications	BSA no added to PCR mixture	Add BSA to a final concentrated of 0.1ug/ml to the PCR mixture.
	Too much DNA Inhibitor PCR reaction	Dilute the DNA elute use in the downstream application if possible
	No-specific bands in downstream PCR	Use hot start Taq DNA Polymerase mixture

A. E.Z.N.A.™ Soil DNA Kit Protocol

Materials to be provided by user

- Microcentrifuge capable of at least 13,000 x g
- Nuclease-free 1.5 ml or 2 ml microfuge tubes
- Water bath equilibrated to 70ºC
- Absolute (96%-100%) ethanol

1. **Weigh 500 mg of glass beads in a 15 ml centrifuge tube, add 0.2-0.5 g soil sample. Add 1 ml Buffer SLX Mlus.** Vortex at maximum speed for 3-5 minute to lyse samples. For the best result, A Mixer Mill, such as Fastprep-24®, Mixer Mill MM 300®, should be used.

2. **Add 100 µl Buffer DS and vortex to mix.**

3. **Incubate at 70°C for 10 min.** Briefly vortex the tube once during the incubation. For some difficult lysis bacterial, Increase the temperature to 90°C.

4. Centrifuge at 3,000 rpm for 3 min at room temperature**. Transfer 800 µL the supernatant into a new 2 ml tube and add 270 µL Buffer SP2.** Mix the sample throughly by vortexing.

5. **Incubate on ice for 5 minutes.** Centrifuge at full speed(13,000 x g) in a microcentrifuge for 5 minutes at 4°C.

6. **Carefully transfer supernatant to a new 2 ml tube and add 0.7 volume of isopropanol.** Mix throughly by inverting tube for 20-30 times. If the soil contains very low DNA, incubate the sample at -20°C for 1 hour.

7. Precipitate DNA by centrifuge at full speed (13,000 x g) for 10 minute at 4°C.

8. **Carefully discard the supernatant and make sure not dislodge the DNA pellet.** Invert the tube on a absorbent paper for 1 minute to drain the liquid. It is not necessary to dry the DNA pellet.

9. **Add 200 µl of Elution Buffer to the tube and vortex for 10 seconds.** Incubate at 65°C for 10-20 minutes to dissolve the DNA pellet.

10. **Add 50-100 µl of HTR Reagent**. Mix throughly by vortexing for 10 seconds.
Note: Completely resuspend HTR Reagent by shaking the bottle before use.

11. **Incubate at room temperature for 2 minutes. Centrifuge at full speed (13,000 x g) for 2 minutes.**

12. **Transfer cleared supernatant to a new 2 ml tube.**
Note: If supernatant still have dark color from soil, perform the HTR treatment again by repeating step 10-12.

13. **Add equal volume of XP2 Buffer, mix by vortexing**. For example: if the sample from step 12 is 250 µl, add 250 µl Buffer XP2.

14. **Apply the sample from step 13 to a HiBind® DNA Column assembled in a 2 ml collection tube (supplied)**. Centrifuge at 10,000 x g for 1 min at room temperature. Discard flow-through liquid and re-use collection tube.

15. **Place the column into the same 2 ml collection tube from previous step and add 300 µl XP2 Buffer**. Centrifuge at 10,000 x g for 1 minute. Discard the flow-through and collection tube.

16. **Place column into a new 2 ml collection tube (supplied) and wash by adding 700 µl SPW Wash Buffer diluted with absolute ethanol.** Centrifuge at 10,000 x g for 1 minute. Discard flow-through liquid and re-use collection tube in next step.
Note: SPW Wash Buffer is provided as a concentrate and must bediluted with absolute ethanol as indicated on the bottle and page 4.

17. **Repeat step 16 with a second 700 µl SPW Wash Buffer.**

18. Discard liquid and re-insert the column to the empty collection tube, **centrifuge the column at full speed (13,000 x g) for 2 min at room temperature**. *This step is critical in removing traces of ethanol that will interfere with downstream applications.*

19. Place column into a clean 1.5 ml microcentrifuge tube (not supplied). **Add 30-100 µl Elution Buffer directly onto the center of HiBind® matrix.** Incubate at 65°C for 10-15 minutes.

20. Centrifuge at full speed (13,000 x g) for 1 min to Elute DNA.

21. **Repeat elution step 19-20 with a second 30-100 µl Elution Buffer**.