omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Soil DNA Kit

D5625-00	5 preps
D5625-01	50 preps
D5625-02	200 preps

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For Research Use Only

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E.Z.N.A.® Soil DNA Kit

Table of Contents

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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 250 mg soil samples can be processed in 60 minutes or up to 1g soil samples in 2.5 hours. 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 next-generation sequencing. 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 to lyse bacteria, yeast, and fungal samples. Humic acid, proteins, polysaccharides, and other contaminants are removed using our propietary cHTR Reagent. Binding conditions are then adjusted and the sample is applied to an HiBind® DNA Mini Column. Two rapid wash steps remove trace contaminants and pure DNA is eluted in low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

New in this Edition:

- cHTR Reagent has replaced HTR Reagent.
- A new protocol is introduced for processing up to 250 mg soil samples.
- Glass Beads have been replaced with Disruptor Tubes, 2 mL tubes pre-filled with glass beads.

Kit Contents

Product Number	D5625-00	D5625-01	D5625-02
Purifications	5 preps	50 preps	200 preps
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
Disruptor Tubes	5	50	200
SLX-Mlus Buffer	6 mL	60 mL	220 mL
DS Buffer	0.6 mL	6 mL	22 mL
P2 Buffer	3 mL	25 mL	60 mL
cHTR Reagent	1.2 mL	12 mL	45 mL
XP1 Buffer	4 mL	40 mL	160 mL
HBC Buffer	4 mL	25 mL	80 mL
DNA Wash Buffer	2 mL	20 mL	3 x 25 mL
Elution Buffer*	3 mL	30 mL	120 mL
User Manual	✓	✓	✓

*Elution Buffer is 10 mM Tris HCl pH 8.5

Storage and Stability

All E.Z.N.A.® Soil DNA Kit components are guaranteed for at least 24 months from the date of purchase when stored as follows. Store cHTR Reagent at 2-8°C. Store all other components at room temperature. Check buffers for precipitates before use. Redissolve any precipitates (except cHTR Reagent) by warming to 55˚C.

Preparing Reagents

1. Dilute HBC Buffer with 100% isopropanol as follows and store at room temperature.

Kit	100% Isopropanol to be Added
D5625-00	1.6 mL
D5625-01	10 mL
D5625-02	32 mL per bottle

2. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D5625-00	8 mL
D5625-01	80 mL
D5625-02	100 mL per bottle

E.Z.N.A.® Soil DNA Kit - Protocol for 100-250 mg samples

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- Vortexer
- 1.5 mL microcentrifuge tubes
- Incubator capable of 70°C
- 100% ethanol
- 100% isopropanol
- Ice bucket

Before Starting:

- Prepare HBC Buffer and DNA Wash Buffer as instructed in the "Preparing Reagents" section on Page 4
- Set a incubator to 70°C
- Heat Elution Buffer to 70°C
- Prepare an ice bucket
- Chill P2 Buffer in an ice bucket

1. Add 100-250 mg soil sample to a Disruptor Tube.

2. Add 725 µL SLX-Mlus Buffer. Vortex at maximum speed for 3-5 minutes to lyse samples.

   Note: For best results, a mixer mill, such as GenoGrinder 2010, Fastprep-24®, or Omni Bead Ruptor should be used.

3. Centrifuge at 500 x *g* for 5 seconds to remove drops of liquid from the lid.

4. Add 72 µL DS Buffer. Vortex to mix thoroughly.

5. Incubate at 70°C for 10 minutes. Briefly vortex the tube once during incubation.

6. Centrifuge at 10,000 x *g* for 5 minutes at room temperature.

7. Transfer 400 µL supernatant into a new 1.5 mL microcentrifuge tube (not provided).

8. Add 135 µL chilled P2 Buffer. Vortex to mix thoroughly.

9. Let sit on ice for 3 minutes.

10. Centrifuge at maximum speed (≥13,000 x *g*) for 1minute.

11. Carefully transfer the supernatant to a new 1.5 mL microcentrifuge tube.

12. Add 200 µL cHTR Reagent. Vortex to mix thoroughly.

    Note: Completely resuspend cHTR Reagent by shaking the bottle before use. It may be necessary to cut the end of the pipette tip to aspirate and dispense cHTR Reagent.

13. Let sit at room temperature for 2 minutes.

14. Centrifuge at maximum speed for 1 minute.

15. Transfer cleared supernatant (~500 µL) to a new 1.5 mL microcentrifuge tube.

    Note: If supernatant still has a dark color from the soil, repeat Steps 12-14 for a second cHTR Reagent step. This will require additional cHTR Reagent that can be purchased separately.

16. Add an equal volume XP1 Buffer. Vortex to mix thoroughly.

17. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube (provided).

18. Transfer up to 700 µL sample from Step 16 to the HiBind® DNA Mini Column.

19. Centrifuge at 10,000 x *g* for 1 minute at room temperature.

20. Discard the filtrate and reuse the Collection Tube.

21. Repeat Steps 18-20 until all the lysate from Step 16 has passed through the HiBind® DNA Mini Column.

22. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see the "Preparing Reagents" section on Page 4 for instructions.

23. Centrifuge at 10,000 x *g* for 1 minute.

24. Discard the filtrate and the Collection Tube.

25. Transfer the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

26. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see the "Preparing Reagents" section on Page 4 for instructions.

27. Centrifuge at 10,000 x *g* for 1 minute.

28. Discard the filtrate and reuse the Collection Tube.

29. Repeat Steps 26-28 for a second DNA Wash Buffer wash step.

30. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes at room temperature.

    Note: This step is critical in removing residual ethanol that may interfere with downstream applications.

31. Transfer the HiBind® DNA Mini Column into a clean 1.5 mL microcentrifuge tube.

32. Add 50-100 µL Elution Buffer heated to 70°C directly onto the center of HiBind® matrix.

33. Let sit at room temperature for 1-2 minutes.

34. Centrifuge at maximum speed for 1 minute.

35. Take the filtrate from Step 34 and place onto the center of the same HiBind® DNA Mini Column used in the procedure.

36. Let sit at room temperature for 1 minute.

37. Centrifuge at maximum speed for 1 minute.

38. Store eluted DNA at -20°C.

E.Z.N.A.® Soil DNA Kit - Protocol for 250-1,000 mg samples

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- Centrifuge with rotor for 15 mL centrifuge tubes
- Vortexer
- 1.5 mL and 2 mL microcentrifuge tubes
- 15 mL centrifuge tubes
- Incubator capable of 70°C
- 100% ethanol
- 100% isopropanol
- Ice bucket

Before Starting:

- Prepare HBC Buffer and DNA Wash Buffer as instructed in the "Preparing Reagents" section on Page 4
- Set a incubator to 70°C
- Heat Elution Buffer to 70°C
- Prepare an ice bucket
- Chill P2 Buffer in an ice bucket

1. Transfer the glass beads from a Disruptor Tube to a 15 mL centrifuge tube (not provided).

2. Add 0.2-1 g soil sample to the 15 mL centrifuge tube.

3. Add 1 mL SLX-Mlus Buffer. Vortex at maximum speed for 3-5 minutes to lyse samples.

   Note: For best result, a mixer mill, such as GenoGrinder 2010, Fastprep-24®, or Mixer Mill MM 300® should be used.

4. Add 100 µL DS Buffer. Vortex to mix thoroughly.

5. Incubate at 70°C for 10 minutes. Briefly vortex the tube once during incubation.

6. Centrifuge at 3,000 rpm for 3 minutes at room temperature.

7. Transfer 800 µL supernatant into a new 2 mL microcentrifuge tube (not provided).

8. Add 270 µL chilled P2 Buffer. Vortex to mix thoroughly.

9. Let sit on ice for 5 minutes.

10. Centrifuge at maximum speed (≥13,000 x *g*) for 5 minutes.

11. Carefully transfer the supernatant to a new 2 mL microcentrifuge tube.

12. Add 0.7 volumes 100% isopropanol. Mix thoroughly by inverting the tube 20-30 times.

    Note: If the soil contains very low DNA, incubate the sample at -20°C for 1 hour.

13. Centrifuge at maximum speed for 10 minutes.

14. Carefully aspirate and discard the supernatant. Do not disturb the DNA pellet.

15. Invert the tube on absorbent paper for 1 minute to drain the liquid.

    Note: It is not necessary to dry the DNA pellet.

16. Add 200 µL Elution Buffer. Vortex for 10 seconds.

17. Incubate at 70°C for 10-20 minutes to dissolve the DNA pellet.

18. Add 100 µL cHTR Reagent. Vortex to mix thoroughly.

    Note: Completely resuspend cHTR Reagent by shaking the bottle before use. It may be necessary to cut the end of the pipette tip to aspirate and dispense cHTR Reagent.

19. Let sit at room temperature for 2 minutes.

20. Centrifuge at maximum speed for 2 minutes.

21. Transfer the cleared supernatant to a new 2 mL microcentrifuge tube.

    Note: If supernatant still has a dark color from the soil, repeat Steps 18-20 for a second cHTR Reagent step. This will require additional cHTR Reagent that can be purchased separately.

22. Add an equal volume XP1 Buffer. Vortex to mix thoroughly.

23. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube (provided).

24. Transfer the sample from Step 22 to the HiBind® DNA Mini Column.

25. Centrifuge at 10,000 x *g* for 1 minute at room temperature.

26. Discard the filtrate and reuse the Collection Tube.

27. Add 500 µL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see the "Preparing Reagents" section on Page 4 for instructions.

28. Centrifuge at 10,000 x *g* for 1 minute.

29. Discard the filtrate and the Collection Tube.

30. Transfer the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

31. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see the "Preparing Reagents" section on Page 4 for instructions.

32. Centrifuge at 10,000 x *g* for 1 minute.

33. Discard the filtrate and reuse the Collection Tube.

34. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes at room temperature.

    Note: This step is critical in removing residual ethanol that may interfere with downstream applications.

35. Transfer the HiBind® DNA Mini Column into a clean 1.5 mL microcentrifuge tube.

36. Add 50-100 µL Elution Buffer heated to 70°C directly onto the center of HiBind® membrane.

37. Let sit at room temperature for 1-2 minutes.

38. Centrifuge at maximum speed for 1 minute.

39. Take the filtrate from Step 38 and place onto the center of the same HiBind® DNA Mini Column used in the procedure.

40. Let sit at room temperature for 1 minute.

41. Centrifuge at maximum speed for 1 minute.

42. Store eluted DNA at -20°C.

E.Z.N.A.® Soil DNA Kit - Purification of DNA isolated using other Methods

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- 1.5 mL and 2 mL microcentrifuge tubes
- Incubator capable of 70°C
- 100% ethanol
- 100% isopropanol
- Vortexer
- Ice bucket

Before Starting:

- Prepare HBC Buffer and DNA Wash Buffer as instructed in the "Preparing Reagents" section on Page 4
- Set a incubator to 70°C and heat Elution Buffer to 70°C
- Prepare an ice bucket and chill P2 Buffer

1. Adjust the volume of the DNA sample to 200 µL with Elution Buffer.

2. Add 100 µL cHTR Reagent. Vortex to mix thoroughly.

   Note: Completely resuspend cHTR Reagent by shaking the bottle before use. It may be necessary to cut the end of the pipette tip to aspirate and dispense cHTR Reagent.

3. Let sit at room temperature for 2 minutes.

4. Centrifuge at ≥13,000 x *g* for 2 minutes.

5. Transfer cleared supernatant to a new 2 mL microcentrifuge tube (not provided).

   Note: If supernatant still has a dark color from the soil, repeat Steps 2-4 for a second cHTR Reagent step. This will require additional cHTR Reagent that can be purchased separately.

6. Follow Steps 16-38 beginning on Page 6 of the E.Z.N.A.® Soil DNA Kit - Protocol for 100-250 mg samples.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, **at 1-800-832-8896.**

Problem	Cause	Solution
A260/230 ratio is low	Inefficient elimination of inhibitory compounds	Repeat with a new sample, be sure to mix the sample with cHTR Reagent thoroughly.
	Salt contamination	Make sure the column is dried before the elution. Wash the column with extra DNA Wash Buffer.
Low DNA Yield or no DNA Yield	Poor homogenization of sample	Repeat the DNA isolation with a new sample, be sure to vortex the sample with SLX-Mlus and glass beads thoroughly.
	DNA washed off.	DNA Wash Buffer must be diluted with 100% ethanol before use.
	Column matrix lost binding capacity during storage	Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x *g* for 30 seconds. Discard the filtrate.
Problems in downstream applications	BSA not added to PCR mixture	Add BSA to a final concentration of 0.1 µg/mL to the PCR mixture.
	Too much DNA inhibits PCR reactions	Dilute the DNA used in the downstream application if possible.
	Non-specific bands in downstream PCR	Use hot-start Taq polymerase mixture.
	Inhibitory substance in the eluted DNA.	Check the $A_{260}/_{230}$ ratio. Dilute the elute to 1:50 if necessary
	Residual ethanol in the elute	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	Clogged column	Check the centrifugal force and increase the time of centrifugation

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
P2 Buffer, 60 mL	PD077
DNA Wash Buffer, 40 mL	PDR044
Elution Buffer, 100 mL	PDR048
cHTR Reagent, 50 mL	PD089

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Notes: