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Introduction

The E.Z.N.A.® Mag-Bind Soil DNA Kit allows rapid and reliable isolation of high-quality total DNA from fresh and frozen soil samples. Up to 1 g of soil samples can be processed in less than 60 minutes. The system combines the reversible nucleic acid-binding properties of Mag-Bind® particles with the efficiency of HTR Reagents to eliminate humic acid, polysaccharides, phenolic compounds, and enzyme inhibitors 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 degrade DNA and inhibit downstream enzymatic reactions. E.Z.N.A.® Mag-Bind Soil DNA Kit uses a unique HTR Reagent and PSP Buffer that can remove inhibitory substances from soil samples.

If using the E.Z.N.A.® Mag-Bind Soil DNA Kit for the first time, please read this booklet to become familiar with the procedures. Frozen or fresh soil samples are homogenized and then lysed in a specially formulated buffer containing detergent. Proteins, polysaccharides, and cellular debris are subsequently precipitated with PSP Buffer after a heat-freeze step. Contaminants are further removed by HTR reagent by a quick centrifuge step. Binding conditions are then adjusted and the DNA is will be selectively bind to the surface of Mag-Bind® Particles. Two rapid wash steps remove trace contaminants, and pure DNA is eluted in DNA Elution Buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

Most components of the E.Z.N.A.® Mag-Bind Soil DNA Kit should be stored at 22°C-25°C. Mag-Bind® Particles Solution should be stored at 4° C for long-term use. During shipment or storage in cool ambient conditions, precipitates may form in Buffer SLX. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Kit Contents

Product Number	M5635-00	M5635-01	M5635-02
Purification	5 Preps	50 Preps	200 Preps
Mag-Bind Particle B	60 µl	550 µl	2.2 ml
Buffer SLX Mlus	6 ml	60 ml	220 ml
Buffer DS	0.6 ml	6 ml	22 ml
P2 Buffer	2.0 mL	20 mL	75 mL
HTR Reagent	1.2 mL	12 mL	45 mL
MGB Binding Buffer	2 mL	5 mL	25 mL
SPM Wash Buffer	3 mL	30 mL	60 mL
RNase A	12 µl	110 µL	420 µL
Glass Beads	1.2 g	12 g	45 g
Elution Buffer	1.5 mL	12 mL	80 mL
Instruction Booklet	1	1	1

Materials to be provided by user

- Microcentrifuge capable of at least 14,000 x g
- Nuclease-free 1.5 mL or 2 mL microfuge tubes
- 15 mL Centrifuge Tubes
- Centrifuge capable of at least 3,000 x g
- Water bath equilibrated to 65ºC
- 2-mercaptoethanol
- Absolute (96%-100%) ethanol
- RNase A stock solution at 20 mg/mL
- PBS Buffer

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.® Mag-Bind Soil DNA Kit protocol.

- Dilute SPM Buffer with absolute ethanol as follows and store at room temperature.

M5635-00	Add 7 mL absolute (96%-100%) ethanol.
M5635-01	Add 70 mL (96%-100%) ethanol to each bottle.
M5635-02	Add 140 mL (96%-100%) ethanol to each bottle.

- Dilute MGB Binding Buffer with absolute ethanol as follows and store at room temperature:

- • Add 10µl of 2-mercaptoethanol per 1 mL of Buffer SLX. This mixture can be stored at room temperature for 2 weeks.
- Preheat a waterbath to 65°C

M5635-00	Add 8 mL absolute (96%-100%) ethanol
M5635-01	Add 20 mL absolute (96%-100%) ethanol
M5635-02	Add 100 mL absolute (96%-100%) ethanol

Mag-Bind® Soil DNA Kit Protocol Using Magnetic stand

1. Weigh 500 mg of glass beads in a 15 ml centrifuge tube, add 0.2-1.0 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. Mix sample twice during incubation by vortexing the tube. Optional: For isolation of DNA from gram positive bacteria, do a second incubation at 95°C for 2 minutes..

4. Add 335µl of Buffer P2 Buffer. Mix throughly by vortexing for 30 seconds.

5. Incubate the sample on ice for 5 minutes.

6. Centrifuge the sample at 3,000 - 5,000 x g for 7 minutes.

7. Carefully transfer supernatant to a new 2.0 mL microfuge tube making sure not to disturb the pellet or transfer any debris.

   Note: Do not transfer more than 1 mL of supernatant

8. Add equal volume of isopropanol and mix throughly by invert

the tube 5-10 times.

9. Centrifuge at full speed (>13,000 x g) for 10 minutes at room temperature.

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

11. Add 200µl of Elution Buffer to the tube and vortex for 10 seconds. Incubate at 65C for 10-20 minutes to dissolve the DNA pellet. Centrifuge briefly to collect any liquid drop from the tube cap.

12. Vigorously mix the bottle of the HTR reagent for 30 seconds to ensure the particles are throughly resuspended. Add 100µl of HRT reagent and mix throughly by vortexing for 10 seconds.

    Important: HTR reagent must be throughly suspended before being dispense from bottle. Tip: Use 1ml pipettor and cut off the end of 1ml tip to make it easier for pipetting the HTR reagent.

13. Incubate at room temperature for 2 minutes.

14. Centrifuge at full speed (>13,000 x g) for 2 minutes.

15. Transfer cleared supernatant to a new 1.5 ml tube. Note: If the supernatant still shows dark color from soil at this point, perform the HTR extraction again by repeating step 11-14.

16. Optional: If RNA-free DNA is required, add 2µl RNase A (25mg/ml) and mix throughly by vortexing for 10 seconds. Incubate at 37°C for 1 minute.

16.. Add one volume of MGB Binding Buffer follow by 10 µL of Mag-Bind® Particles Solution B. Mix throughly by pipetting up and down for 10-20 times.

    Note: 1. Dilute MGB Buffer with absolute ethanol before use. See Page 4 or bottle label for instructions. 2. Mag-Bind® Particles Solution B will bead together in its container after several hours. It must be fully suspended by shaking or vortexing before use. (IMPORTANT)

17. Incubate the sample at room temperature for 5 minutes.

18. Place the tube on a magnetic separation device suitable for 1.5 mL tube to magnetize the Mag-Bind® particles. Carefully remove and discard the cleared supernatant.

19. Remove the tube containing the Mag-Bind® particles from the magnetic separation device.

20.. Add 500 µL of SPM Wash Buffer diluted with ethanol into the tube.

21. Resuspend Mag-Bind® particles pellet by vortexing or pipetting. Incubate 2 minutes at room temperature.

22 Place the tube onto a magnetic separation device to magnetize the Mag-Bind® particles. Carefully remove and discard the cleared supernatant.

23 Wash the Mag-Bind particles with SPM one more time by repeating step 20-22.

24 After remove the supernatant, air dry the magnetic beads by invert the tube on a absorbent paper for 5 minutes. Remove any residue liquid from tube with pipettor..

25 Add 50-100 ul Elution Buffer or water prewarmed to 65°C to the tube. Incubate the tube at Resuspend Mag-Bind® particles by vortexing or pipetting up and down for 20 times.

26. Place the tube onto a magnetic separation device to magnetize the Mag-Bind® particles.

27. Transfer the cleared supernatant containing purified DNA to a new 1.5 mL tube.

Mag-Bind® Soil DNA Kit Protocol Using Centrifuge

1. Weigh 200 mg of glass beads in a 15 mL centrifuge tube, add 0.2-1 g soil sample. Add 1000 µL Buffer SLX. Vortex at maximum speed for 3 minute or until the sample is throughly homogenized.

2. Incubate at 70°C for 10 min. Mix sample twice during incubation by vortexing the tube. Optional: For isolation of DNA from gram positive bacteria, do a second incubation at 95°C for 2 minutes..

3. Add 335 µl of Buffer P2 Buffer. Mix throughly by vortexing for 30 seconds.

4. Incubate the sample on ice for 5 minutes.

5. Centrifuge the sample at 3,000 - 5,000 x g for 7 minutes.

6. Carefully transfer supernatant to a new 2.0 mL microfuge tube making sure not to disturb the pellet or transfer any debris.

Note: Do not transfer more than 1 mL of supernatant

7. Add equal volume of isopropanol and mix throughly by invert the tube 5-10 times.

8. Centrifuge at full speed (>13,000 x g) for 10 minutes at room temperature.

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

10. Add 200µl of Elution Buffer to the tube and vortex for 10 seconds. Incubate at 65°C for 10-20 minutes to dissolve the DNA pellet. Centrifuge briefly to collect any liquid drop from the tube cap.

11. Vigorously mix the bottle of the HTR reagent for 30 seconds to ensure the particles are throughly resuspended. Add 100µl of HTR reagent and mix throughly by vortexing for 10 seconds.

    Important: HTR reagent must be throughly suspended before being dispense from bottle. Tip: Use 1ml pipettor and cut off the end of 1ml tip to make it easier for pipetting the HTR reagent.

12. Incubate at room temperature for 2 minutes.

13. Centrifuge at full speed (>13,000 x g) for 2 minutes.

14. Transfer cleared supernatant to a new 1.5 ml tube. Note: If the supernatant still shows dark color from soil at this point, perform the HTR extraction again by repeating step 11-14.

15. Optional: If RNA-free DNA is required, add 2µl RNase A and mix throughly by vortexing for 10 seconds. Incubate at 37°C for 1 minute.

16.. Add one volume of MGB Binding Buffer follow by 10 µL of Mag-Bind® Particles Solution B. Mix throughly by pipetting up and down for 10-20 times.

    Note: 1. Dilute MGB Buffer with absolute ethanol before use. See Page 4 or bottle label for instructions. 2. Mag-Bind® Particles Solution B will bead together in its container after several hours. It must be fully suspended by shaking or vortexing before use. (IMPORTANT)

17. Incubate the sample at room temperature for 5 minutes.

18. Centrifuge at 10,000 x g for 1 minute.. Carefully remove and discard the cleared supernatant.

20.. Add 500 µL of SPM Wash Buffer diluted with ethanol into the tube.

21. Resuspend Mag-Bind® particles pellet by vortexing or pipetting. Incubate 2 minutes at room temperature.

22 Centrifuge at 10,000 x g for 1 minute. Carefully remove and discard the cleared supernatant.

23 Wash the Mag-Bind particles with SPM one more time by repeating step 20-22.

24 After removing the supernatant, air dry the magnetic beads by invert the tube on a absorbent paper for 5 minutes. Remove any residue liquid from tube with pipettor..

25 Add 50-100 ul Elution Buffer or water prewarmed to 65 to the tube. Incubate the tube at Resuspend Mag-Bind® particles by vortexing or pipetting up and down for 20 times.

26. Centrifuge at 10,000 x g for 1 minute.

27. Transfer the cleared supernatant containing purified DNA to a new 1.5 mL tube.

Mag-Bind® Soil DNA Clean-up Protocol Using Magnet

The following protocol is designed for clean-up the DNA purified with some in-house purification methods or other commercial products.

1. Transfer 400µl of crude DNA sample to a 1.5 ml tube. If the sample volume is less than 400ul, add Elution Buffer (supplied with this kit) or TE Buffer to bring the volume to 400µl.

2. Add 400µl of HTR Reagent to the tube and mix throughly by vortexing for 20 seconds.

3. Incubate at room temperature for 3 minutes.

4. Centrifuge at full speed (14,000-20,000 x g) for 2 minutes.

5. Transfer the supernatant to a clean new 1.5 ml centrifuge tube, make sure not to disturb the pellet.

6. Add equal volume of MGB Binding Bufer followed by 10µl of Mag-Bind Particle solution B. Mix throughly by vortexing or pipetting.

7. Incubate the sample at room temperature for 5 minutes.

8. Place the tube on a magnetic separation device suitable for 1.5 mL tube to magnetize the Mag-Bind® particles. Carefully remove and discard the cleared supernatant.

9. Remove the tube containing the Mag-Bind® particles from the magnetic separation device.

10. Add 500 µL of SPM Wash Buffer diluted with ethanol into the tube.

11. Resuspend Mag-Bind® particles pellet by vortexing or pipetting. Incubate 2 minutes at room temperature.

12. Place the tube onto a magnetic separation device to magnetize the Mag-Bind® particles. Carefully remove and discard the cleared supernatant.

13. Wash the Mag-Bind particles with SPM one more time by repeating step 10-12.

14. After remove the supernatant, air dry the magnetic beads by invert the tube on a absorbent paper for 5 minutes. Remove any residue liquid from tube with pipettor.

15. Add 50-100 ul Elution Buffer or water prewarmed to 65°C to the tube. Resuspend Mag-Bind® particles by vortexing or pipetting up and down for 20 times.

16. Place the tube onto a magnetic separation device to magnetize the Mag-Bind® particles.

17. Transfer the cleared supernatant containing purified DNA to a new 1.5 mL tube.

Mag-Bind® Soil DNA Clean-up Protocol Using Centrifuge

The following protocol is designed for clean-up the DNA purified with some in-house purification methods or other commercial products.

1. Transfer 400µl of crude DNA sample to a 1.5 ml tube. If the sample volume is less than 400ul, add Elution Buffer (supplied with this kit) or TE Buffer to bring the volume to 400µl.

2. Add 400µl of HTR Reagent to the tube and mix throughly by vortexing for 20 seconds.

3. Incubate at room temperature for 3 minutes.

4. Centrifuge at full speed (14,000-20,000 x g) for 2 minutes.

5. Transfer the supernatant to a clean new 1.5 ml centrifuge tube, make sure not to disturb the pellet.

6. Add equal volume of MGB Binding Bufer followed by 10µl of Mag-Bind Particle solution B. Mix throughly by vortexing or pipetting.

7. Incubate the sample at room temperature for 5 minutes.

8. Centrifuge at 14,000 x g for 3 minutes to collet the Mag-Bind Particles. Carefully remove and discard the cleared supernatant.

9. Add 500 µL of SPM Wash Buffer diluted with ethanol into the tube.

10. Resuspend Mag-Bind® particles pellet by vortexing or pipetting.

11. Incubate 2 minutes at room temperature.

12. Centrifuge at 14,000 x g for 3 minutes to to collet the Mag-Bind Particles. . Carefully remove and discard the cleared supernatant.

13. Add 500 µL of SPM Wash Buffer diluted with ethanol into the tube.

14. Resuspend Mag-Bind® particles pellet by vortexing or pipetting.

15. Incubate 2 minutes at room temperature.

16. Centrifuge at 14,000 x g for 1 minute to to collet the Mag-Bind Particles. . Carefully remove and discard the cleared supernatant.

17. After remove the supernatant, air dry the magnetic beads by invert the tube on a absorbent paper for 5 minutes. Remove any residue liquid from tube with pipettor.

18. Add 50-100 ul Elution Buffer or water to the tube. Resuspend Mag-Bind® particles by vortexing or pipetting up and down for 20 times.

19. Place the tube onto a magnetic separation device to magnetize the Mag-Bind® particles.

20. Transfer the cleared supernatant containing purified DNA to a new 1.5 mL tube.

Trouble Shooting Guide

Problem	Cause	Suggestions
Low DNA yield	Incomplete disruption of starting material.	Repeat the experiment with new sample, make sure the sample are completely interrupted and lysed.
	Sample stored incorrectly	Store the sample at -20°C
	Loss the Mag-Bind particle during operation	Carefully avoid remove the Mag-Bind® particles during aspiration
	DNA remains bound to Mag-Bind® Particles	Increase elution volume and incubate at 65°C for 5 min elution
	DNA washed off.	Dilute MGB Binding Buffer and SPM Wash Buffer by adding appropriate volume of absolute ethanol prior to use (page 3).
Problems in downstream applications	Ethanol carry-over	Wash Buffer must be at room temperature.
		Dry the Mag-Bind® particle before elution.
	Add BSA to a final concentration of 0.1µg/mL to the PCR mixture.	Add BSA to a final concentration of 0.1µg/mL to the PCR mixture.
A260/280 ratio is low	**inefficient elimination of inhibitory compounds**	**Repeat with a new sample, be sure to mix HTR Reagent throughly before use**