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Introduction

The Mag-Bind? Soil DNA Kit allows rapid and reliable isolation of high-quality total DNA from various soil samples. Up to 0.5 gram of soil samples can be processed in less than 60 minutes. The system combines the reversible nucleic acid-binding properties of MagSi? particles with the efficiency of cHTR 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.

Storage and Stability

Most components of the Mag-Bind? Soil DNA Kit should be stored at 22°C-25°C. MagSi? Particles Solution and cHTR should be stored at 4°C for long-term use. During shipment or storage in cool ambient conditions, precipitates may form in Buffer DS. 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
MagSi? Particles	250 µl	2 x 1.1 ml	9 ml
Buffer SLX Mlus	6 ml	60 ml	220 ml
Buffer DS	0.6 ml	6 ml	22 ml
Buffer SP2	2 ml	20 ml	50 ml
cHTR Reagent	2 ml	15 ml	50 ml
Binding Buffer	6 ml	60 ml	220 ml
Buffer PHB	5 ml	60 ml	250 ml
SPM Wash Buffer	2 ml	2 x 20 ml	2 x 40 ml
Glass Beads	3 g	30 g	120 g
Elution Buffer	1.8 ml	20 ml	100 ml
Instruction Booklet	1	1	1

Before Starting

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

M5635-00	Add 8 ml absolute (96%-100%) ethanol.
M5635-01	Add 80 ml (96%-100%) ethanol to each bottle.
M5635-02	Add 160 ml (96%-100%) ethanol to each bottle.

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
- Water bath equilibrated to 65ºC
- Absolute (96%-100%) ethanol

Please read the entire booklet to become familiar with the Mag-Bind? Soil DNA Kit protocol.

Mag-Bind? Soil DNA Kit Protocol

1. **Weigh 500 mg of glass beads in a 2 ml centrifuge tube, add 0.25-0.5 g soil sample. Add 0.7 ml Buffer SLX Mlus.** Vortex at maximum speed for 5-10 minute to lyse samples. For the best result, A Mixer Mill, such as Fastprep-24?, Mixer Mill MM 300?, should be used.

2. **Add 80 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 13,000 x g for 5 min.

5. **Transfer 600 µl the supernatant into a new 2 ml tube and add 200 µl Buffer SP2.** Vortex to mix well.

6. **Add 100 µl cHTR Reagent**. Mix throughly by vortexing for 10 seconds. **Incubate on ice for 5 minutes.** Centrifuge at 13,000 x g in a microcentrifuge for 5 minutes.

7. **Transfer 400 µl of the cleared supernatant to a new 1.5 ml tube.**

8. **Add 450 µl Binding Buffer and 40 µl of MagSi? Particles to the sample.** Invert to mix well. Incubate at room temperature for 2 minutes.

9. **Place the tube on a magnetic separation device suitable for 2 ml tube to magnetize the MagSi? Particles**. Carefully remove and discard the cleared supernatant.

10. **Remove the tube containing the MagSi? Particles from the magnetic separation device**.

11. **Add 500 µl Binding Buffer into the tube**. Resuspend MagSi? Particles pellet by vortexing.

12. Place the tube onto a magnetic separation device to magnetize the MagSi[?] Particles. Carefully remove and discard the cleared supernatant. **Add 1000 µl Buffer PHB into the tube**. Resuspend MagSi[?] Particles pellet by vortexing.

13. Place the tube onto a magnetic separation device to magnetize the MagSi[?] Particles. Carefully remove and discard the cleared supernatant.

14. **Add 1000 µl of SPM Wash Buffer diluted with ethanol into the tube.** Resuspend Mag-Bind[?] particles pellet by vortexing.

15. Place the tube onto a magnetic separation device to magnetize the MagSi[?] Particles. Carefully remove and discard the cleared supernatant.

16. Wash MagSi particles with SPM Wash Buffer one more time by repeating step 14-15.

17. **After remove the supernatant, air dry the magnetic beads by invert the tube on a absorbent paper for 15 minutes**. Remove any residue liquid from tube with pipettor.

18. **Add 50-100 ul Elution Buffer or water to the tube.** Incubate the tube at Resuspend MagSi[?] Particles by vortexing. Incubate at 65°C for 10 minutes if maxium DNA yield is desired.

19. Place the tube onto a magnetic separation device to magnetize MagSi[?] Particles.

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

Mag-Bind[?] Soil DNA Clean-up Protocol

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

1. **Transfer 200µl of crude DNA sample to a 1.5 ml tube.** If the sample volume is less than 200ul, add Elution Buffer (supplied) or TE Buffer to bring the volume to 200µl.

2. **Add 100µl of cHTR 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 250 µl of the supernatant to a clean new 1.5 ml centrifuge tube, make sure not to disturb the pellet.

6. **Add 300 µl Binding Buffer and 40 µl of MagSi Particles to the sample.** Invert to mix well. Incubate at room temperature for 2 minutes.

7. **Place the tube on a magnetic separation device suitable for 2 ml tube to magnetize the MagSi[?] Particles**. Carefully remove and discard the cleared supernatant.

8. Remove the tube containing the MagSi[?] Particles from the magnetic separation device. **Add 500 µl Binding Buffer into the tube**. Resuspend MagSi[?] Particles pellet by vortexing.

9. Place the tube onto a magnetic separation device to magnetize the MagSi[?] Particles. Carefully remove and discard the cleared supernatant. Remove the tube containing the MagSi[?] Particles from the magnetic separation device.

10. **Add 1000 µl Buffer PHB into the tube**. Resuspend MagSi$^{?}$ Particles pellet by vortexing.

11. Place the tube onto a magnetic separation device to magnetize the MagSi$^{?}$ Particles. Carefully remove and discard the cleared supernatant.

12. **Add 1000 µl of SPM Wash Buffer diluted with ethanol into the tube.** Resuspend MagSi$^{?}$ Particles pellet by vortexing.

13. Place the tube onto a magnetic separation device to magnetize the MagSi$^{?}$ Particles. Carefully remove and discard the cleared supernatant.

14. Wash MagSi$^{?}$ Particles with SPM Wash Buffer one more time by repeating step 12-13.

15. **After remove the supernatant, air dry the MagSi$^{?}$ Particles by invert the tube on a absorbent paper for 15 minutes**. Remove any residue liquid from tube with pipettor.

16. **Add 50-100 ul Elution Buffer or water to the tube.** Incubate the tube at resuspend MagSi$^{?}$ Particles by vortexing. Incubate at 65°C for 10 minutes if maxium DNA yield is desired.

17. Place the tube onto a magnetic separation device to magnetize the MagSi$^{?}$ Particles.

18. 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 MagSi$^{?}$ Particles during operation	Carefully avoid remove the MagSi$^{?}$ Particles during aspiration
	DNA remains bound to MagSi$^{?}$ Particles	Increase elution volume and incubate at 65°C for additional 10 min elution
	DNA washed off.	Dilute 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 MagSi$^{?}$ Particles 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 cHTR Reagent throughly before use
Eluted DNA still show color	inefficient elimination of inhibitory compounds	Start with a new sample, use extra cHTR or duplicate the cHTR extraction step.