Mag-Bind® Soil DNA Kit

M5635-00	5 preps
M5635-01	50 preps
M5635-02	200 preps

January 2013

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Table of Contents

Manual Revision: January 2013

OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction and Overview

The Mag-Bind Soil DNA Kit allows rapid and reliable isolation of high-quality genomic DNA from various soil samples. Up to 0.25 grams of soil samples can be processed in less than 60 minutes. The system combines the Mag-Bind technology with HTR reagent 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.

If using the Mag-Bind 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. 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.

New in this Edition:

- The latest protocol has been updated and enhanced to maximize protocol quality and readability.

Kit Contents

Product Number	M5635-00	M5635-01	M5635-02
Preparations	5 preps	50 preps	200 preps
Mag-Bind® Particles CND	120 µL	1.1 mL	4.4 mL
Glass Beads	3 g	30 g	110 g
HTR Reagent	1.2 mL	12 mL	45 mL
SLX-Mlus Buffer	6 mL	60 mL	220 mL
DS Buffer	0.6 mL	6 mL	22 mL
SP2 Buffer	2.0 mL	20 mL	75 mL
XP2 Buffer	5 mL	30 mL	110 mL
VHB Buffer	2.2 mL	22 mL	88 mL
Elution Buffer	1.5 mL	20 mL	80 mL
SPM Wash Buffer	3 mL	30 mL	60 mL
Binding Enhancer	55 µL	300 µL	1.1 mL
RNase A	12 µL	110 µL	420 µL
User Manual	✓	✓	✓

Storage and Stability

All of the Mag-Bind® Soil DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles CND, HTR Reagent, RNase A, and Binding Enhancer should be stored at 2-8°C for long-term use. All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

- Dilute SPM Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M5635-00	7 mL
M5635-01	70 mL
M5635-02	140 mL per bottle

- Dilute VHB Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M5635-00	2.8 mL
M5635-01	28 mL
M5635-02	112 mL

Mag-Bind® Soil DNA Kit Protocol

Materials and Equipment to be Supplied by User:

- Refrigerated microcentrifuge capable of at least 13,000 x *g*
- 1.5 mL microcentrifuge tubes
- 2 mL microcentrifuge tubes
- Incubator capable of 70°C
- 100% Ethanol
- 15 mL centrifuge tubes
- Centrifuge with rotor for 15 mL centrifuge tubes
- Vortexer
- Magnetic separation device for 1.5 mL/2.0 mL microcentrifuge tubes

Before Starting:

- Set an incubator to 70°C
- Heat Elution Buffer to 70°C
- Set an incubator or water bath to 95°C (optional for gram-positive bacteria)
- Prepare ice bucket
- Cool a microcentrifuge to 4°C

1. Add 500 mg glass beads to a 15 mL centrifuge tube.

2. Add 0.25 g soil sample.

3. Add 0.6 mL SLX-Mlus Buffer. Vortex at maximum speed for 3-5 minutes to lyse the samples. For the best result, a Mixer Mill, such as GenoGrinder 2010, Fastprep-24®, Mixer Mill MM 300®, should be used.

4. Add 60 µL DS Buffer. Vortex to mix.

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

Optional: For DNA isolation from gram-positive bacteria, do a second incubation at 95°C for 2 minutes.

6. Centrifuge at 13,000 x *g* for 3 minutes at room temperature.

7. Transfer 400 µL supernatant into a new 2 mL microcentrifuge tube.

8. Add 133 µL P2 Buffer. Vortex to mix thoroughly.

9. Add 133 µL HTR Reagent. Vortex to mix thoroughly.

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

10. Let sit on ice for 5 minutes.

11. Centrifuge at 13,000 x *g* for for 5 minutes at 4°C.

12. Carefully transfer supernatant to a new 2 mL microcentrifuge tube.

13. Add 0.5 volumes XP2 Buffer, 20 µL Mag-Bind® Particles CND, and 5 µL Binding Enhancer. Pipet up and down 10 times to mix thoroughly.

14. Let sit at room temperature for 5 minutes.

15. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

16. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

17. Remove the tube from magnetic separation device.

18. Add 500 µL XP2 Buffer. Vortex or pipet up and down to completely resuspend the Mag-Bind® Particles CND.

19. Let sit at room temperature for 2 minutes.

20. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

21. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

22. Remove the tube from magnetic separation device.

23. Add 500 µL VHB Buffer. Vortex or pipet up and down to completely resuspend the Mag-Bind® Particles CND.

24. Let sit at room temperature for 2 minutes.

25. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

26. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

27. Remove the tube from magnetic separation device.

28. Add 500 µL SPM Wash Buffer. Vortex or pipet up and down to completely resuspend the Mag-Bind® Particles CND.

    Note: SPM Wash Buffer must be diluted with ethanol before use. Please see Page 4 for instructions.

29. Let sit at room temperature for 2 minutes.

30. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

31. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

32. Repeat Steps 27-31 for a second SPM Wash Buffer wash step.

33. Leave the tube on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles CND. Remove any residue liquid with a pipettor.

34. Add 50-100 µL Elution Buffer or water heated to 65°C. Vortex or pipet up and down 20 times to completely resuspend the Mag-Bind® Particles CND.

35. Let sit at room temperature for 5 minutes.

36. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

37. Transfer the cleared supernatant to a new 1.5 mL microcentrifuge tube.

38. Store at -20°C.

Mag-Bind® Soil DNA Kit Protocol - DNA Purification Protocol

This protocol can be used to further purify DNA that has been isolated using other kits.

Materials and Equipment to be Supplied by User:

- Refrigerated microcentrifuge capable of at least 13,000 x *g*
- 1.5 mL microcentrifuge tubes
- 2 mL microcentrifuge tubes
- Incubator capable of 70°C
- 100% Ethanol
- 15 mL centrifuge tubes
- Centrifuge with rotor for 15 mL centrifuge tubes
- Vortexer
- Magnetic separation device for 1.5 mL/2.0 mL microcentrifuge tubes
- Optional: TE Buffer (pH 8.0)

Before Starting:

- Set an incubator to 70°C
- Heat Elution Buffer to 70°C
- Set an incubator or water bath to 95°C (optional for gram-positive bacteria)
- Prepare ice bucket
- Cool a microcentrifuge to 4°C

1. Dissolve the DNA pellet with 200 µL Elution Buffer or TE Buffer (pH 8.0).

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

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

3. Let sit at room temperature for 2 minutes.

4. Centrifuge at maximum speed (≥13,000 x *g*) for 2 minutes.

5. Carefully transfer the cleared supernatant into a new microcentrifuge tube.

   Note: If the supernatant still has a dark color from the soil, repeat Steps 2-4.

6. Add 0.5 volumes XP2 Buffer, 20 µL Mag-Bind® Particles CND, and 5 µL Binding Enhancer. Pipet up and down 10 times to mix thoroughly.

7. Let sit at room temperature for 5 minutes.

8. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

9. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

10. Remove the tube from magnetic separation device.

11. Add 500 µL XP2 Buffer. Vortex or pipet up and down to completely resuspend the Mag-Bind® Particles CND.

12. Let sit at room temperature for 2 minutes.

13. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

14. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

15. Remove the tube from magnetic separation device.

16. Add 500 µL VHB Buffer. Vortex or pipet up and down to completely resuspend the Mag-Bind® Particles CND.

17. Let sit at room temperature for 2 minutes.

18. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

19. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

20. Remove the tube from magnetic separation device.

21. Add 500 µL SPM Wash Buffer. Vortex or pipet up and down to completely resuspend the Mag-Bind® Particles CND.

    Note: SPM Wash Buffer must be diluted with ethanol before use. Please see Page 4 for instructions.

22. Let sit at room temperature for 2 minutes.

23. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

24. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CND.

25. Repeat Steps 27-31 for a second SPM Wash Buffer wash step.

26. Leave the tube on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles CND. Remove any residue liquid with a pipettor.

27. Add 50-100 µL Elution Buffer or water heated to 65°C. Vortex or pipet up and down 20 times to completely resuspend the Mag-Bind® Particles CND.

28. Let sit at room temperature for 5 minutes.

29. Place the tube on a magnetic separation device to magnetize the Mag-Bind® Particles CND. Let sit at room temperature until the Mag-Bind® Particles CND are completely cleared from solution.

30. Transfer the cleared supernatant to a new 1.5 mL microcentrifuge tube.

31. Store at -20°C.

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
A_{260}/A_{230} ratio is low	Inefficient elimination of inhibitory compounds	Repeat with a new sample. Be sure to mix with HTR Reagent thoroughly. Add 100 µL to cleared supernatant. Vortex to mix. Incubate for 2 minutes. Centrifuge at 13,000 x *g* for 1 minute and transfer cleared supernatant to next step. Do not reuse SP2 Buffer.
	Salt contamination	• Repeat with a new sample. • Make sure the column is dried before the elution. • Repeat SPW Wash Buffer wash step.
Problem	Cause	Solution
A_{260}/A_{280} ratio is high	RNA contamination	Be sure to treat the sample with RNase A according to the protocol.
Problem		Solution
Low DNA Yield or no DNA Yield	Poor sample homogenization	Repeat with a new sample. Be sure to mix the sample with SLX-Mlus thoroughly.
	DNA washed off	
Problem		Solution
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 elute 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}/A_{230} ratio. Dilute the elute to 1:50 if necessary.
	Ethanol residue in the elute	Completely dry the column before elution.
Problem		Solution
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.

Ordering Information

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

Product	Part Number
Magnetic Separation Device for 1.5 mL Microcentrifuge Tubes	MSD-02
DNase/RNase Free Microcentrifuge Tubes, 1.5 mL, 500/pk, 10 pk/cs	SSI-1210-00
Elution Buffer, 100 mL	PDR048
SPM Wash Buffer, 40 mL	PS014
XP2 Buffer (Binding Buffer), 200 mL	PDR040
SP2 Buffer, 60 mL	PD073
RNase A, 5 mL	PD090

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