omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

Mag-Bind® Environmental DNA 96 Kit

M5645-00	1 x 96 preps
M5645-01	4 x 96 preps

Manual Date: July 2020
Revision Number: v9.0

For Research Use Only

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Introduction

The Mag-Bind® Environmental DNA 96 Kit allows rapid and reliable isolation of high-quality DNA from soil and water samples. The Mag-Bind® Environmental DNA 96 Kit can isolate microbial DNA from yeast, fungi, and gram-positive or gram-negative bacteria. Up to ninety-six 100 mg soil samples or 100 mL water samples can be processed in 120 minutes using automated liquid handlers or magnetic processors. Omega Bio-tek's unique cHTR Reagent effectively removes humic acid and other PCR inhibitors allowing for purified DNA to be suitable for PCR, 16S sequencing, whole-genome sequencing, 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 Mag-Bind® Environmental DNA 96 Kit for the first time, please read this booklet to become familiar with the procedure. The system utilizes Omega Bio-tek's E-Z 96 Disruptor Plates C Plus which are pre-aliquoted with ceramic beads of different sizes in a convenient 96-well format for efficient sample homogenization. Humic acid, proteins, polysaccharides, and other contaminants are removed using our proprietary cHTR Reagent. Binding conditions are then adjusted and the sample is mixed with Mag-Bind® Particles RQ for DNA binding. Three 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.

Important: If automating this procedure on a liquid handler or a magnetic processor, please contact your Omega Bio-tek representative for instrument-specific instructions.

New in this Edition:

July 2020
- An optional lysozyme digestion step for improved recovery of DNA from gram-positive bacteria was added to the Water Protocol.

October 2018
- M5645 Mag-Bind® Soil DNA 96 Kit has been modified and renamed Mag-Bind® Environmental DNA 96 Kit.
- In addition to the Soil Protocol, the Mag-Bind® Environmental DNA 96 Kit includes a protocol for DNA purification from water samples.

Kit Contents

Product Number	M5645-00	M5645-01
Purifications	1 x 96 Preps	4 x 96 Preps
E-Z 96 Disruptor Plate C Plus	1	4
96-well Racked Microtubes	1	4
Caps for Racked Microtubes	30	120
SLX-Mlus Buffer	120 mL	460 mL
DS Buffer	40 mL	160 mL
P2 Buffer	15 mL	60 mL
XP1 Buffer	100 mL	360 mL
VHB Buffer	44 mL	3 x 44 mL
Elution Buffer	15 mL	60 mL
Mag-Bind® Particles RQ	2.2 mL	8.8 mL
cHTR Reagent	25 mL	90 mL
RNase A	220 µL	880 µL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® Environmental DNA 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles RQ, cHTR Reagent, and RNase A 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.

Preparing Reagents

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

Kit	100% Ethanol to be Added
M5645-00	56 mL
M5645-01	56 mL per bottle

2. Water Protocol ONLY: Prepare 15 mL centrifuge tubes (not provided) with E-Z 96 Disruptor Plate C Plus as follows:

- Centrifuge the E-Z 96 Disruptor Plate C Plus at ≥ 2,000*g* for 1 minute.
- Take out one of the 8-tube strips containing the beads and cut off one well using scissors or a razor blade.
- Remove the lid of the well and pour the bead contents into a 15 mL centrifuge tube.
- The 15 mL centrifuge tube is now ready for use in the Water Protocol (Page 9).

Mag-Bind® Environmental DNA 96 Kit - Soil Protocol

Important: If automating this procedure on a liquid handler or a magnetic processor, please contact your Omega Bio-tek representative for instrument-specific instructions.

Materials and Equipment to be Supplied by User:

- Centrifuge equipped with swing-bucket rotor and plate adaptor capable of at least 2,000*g*
- Magnetic separation device (Recommended Alpaqua Magnum FLX)
- Incubator capable of 70°C
- 96-well deep-well plates with a 1.2 mL capacity compatible with magnetic separation device used
- 96-well microplates for DNA storage
- Vortexer
- Ice bucket
- 70% ethanol
- 100% ethanol
- Sealing film
- Optional: Incubator capable of 95°C
- Optional: Mixer Mill such as Spex Certiprep GenoGrinder 2010 or Eppendorf Mixmate

Before Starting:

- Set an incubator to 70°C.
- Heat Elution Buffer to 70°C.
- Prepare an ice bucket and prechill P2 Buffer on ice.
- Prepare 70% ethanol for use in Step 24. A minimum of 1 mL is required per sample.
- Prepare VHB Buffer according to the "Preparing Reagents" section on Page 4.
- Optional: for gram-positive bacteria, set an incubator or water bath to 95°C.

1. Briefly centrifuge the E-Z 96 Disruptor Plate C Plus to remove any ceramic beads from the walls of the wells. Uncap the E-Z 96 Disruptor Plate C Plus and save the caps for use in Step 3 below.

2. Add 10-250 mg soil sample.

3. Add 525 µL SLX-Mlus Buffer and 2 µL RNase A. Seal the plate with the caps removed in Step 1.

   Note: SLX-Mlus Buffer and RNase A can be made as a mastermix.

4. Vortex at maximum speed for 3-5 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex Certiprep GenoGrinder 2010 or Eppendorf Mixmate, should be used.

   Note: Complete homogenization is critical for best yields.

5. Centrifuge at 500*g* for 10 seconds.

6. Remove and discard caps. Add 53 µL DS Buffer. Seal the plate with new Caps for Racked Microtubes.

7. Vortex to mix thoroughly.

8. Incubate at 70°C for 10 minutes. Briefly vortex the plate once during incubation.

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

9. Centrifuge at ≥ 2,000*g* for 10 minutes at room temperature.

10. Transfer 200 µL supernatant to a new set of 96-well Racked Microtubes.

11. Add 67 µL prechilled P2 Buffer and 100 µL cHTR Reagent. Seal the plate with new Caps for Racked Microtubes. Vortex to mix thoroughly.

    Note: Prechill P2 Buffer on ice before use. Completely resuspend cHTR Reagent by shaking the bottle before use. If necessary, cut the pipet tip to transfer cHTR Reagent.

12. Centrifuge at ≥ 2,000*g* for 5 minutes.

13. Transfer supernatant to a 96-well deep-well plate (not provided; must be compatible with the magnetic separation device used).

14. Add 1 volume XP1 Buffer and 20 µL Mag-Bind® Particles RQ. Mix thoroughly by pipetting up and down 10 times.

    Note: Mag-Bind® Particles RQ and XP1 Buffer can be prepared as a mastermix prior to use. Prepare only what is needed.

15. Let sit at room temperature for 10 minutes.

    Note: Continuous mixing of samples will increase yields.

16. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles RQ. Let sit for 5 minutes or until Mag-Bind® Particles RQ have completely migrated to the well wall.

    Note: If Mag-Bind® Particles RQ at the top of well are not completely migrating, pipet up and down while plate is on magnet to help move Mag-Bind® Particles RQ at top closer to magnetic field.

17. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

18. Remove the plate containing the Mag-Bind® Particles RQ from the magnetic separation device.

19. Add 500 µL VHB Buffer.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.

20. Vortex for 30-60 seconds at room temperature.

21. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

22. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

23. Remove the plate containing the Mag-Bind® Particles RQ from the magnetic separation device.

24. Add 500 µL 70% ethanol.

25. Vortex for 30-60 seconds at room temperature.

26. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

27. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles RQ.

28. Repeat Steps 23-27 for a second 70% ethanol wash step.

29. Leave the plate on the magnetic separation device. Wait 1 minute. Remove any residual liquid with a pipette.

30. Leave the plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles RQ.

31. Add 50-100 µL Elution Buffer preheated to 70°C.

32. Vortex for 5 minutes at room temperature.

    Note: If constant vortexing is not possible, vortex for 15 seconds every 1-2 minutes for 5 minutes.

33. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

34. Transfer the cleared supernatant containing purified DNA to a clean 96-well microplate (not provided) and seal with sealing film (not provided).

35. Store the DNA at -20°C.

Mag-Bind® Environmental DNA 96 Kit - Water Protocol

Important: If automating this procedure on a liquid handler or a magnetic processor, please contact your Omega Bio-tek representative for instrument-specific instructions.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000*g*
- Centrifuge capable of at least 2,000*g* equipped with adpator for 15 mL centrifuge tubes
- Magnetic separation device (Recommended Alpaqua Magnum FLX)
- Incubator capable of 70°C
- Vacuum with appropriate adapter for filter paper used
- 96-well deep-well plates with a 2.0 mL capacity compatible with magnetic separation device used
- 96-well microplates for DNA storage
- 0.22 µm or 0.45 µm microporous filter paper (Recommended Sterivex filter (Millipore SVGPL10RC) for optimal yields. Other polyethersulfone filters (PES) can result in lower yields)
- Ice bucket
- Vortexer
- Nuclease-free 1.5 mL or 2.0 mL microcentrifuge tubes
- Nuclease-free 15 mL centrifuge tubes
- Lysozyme (50 mg/mL)
- 70% ethanol
- 100% ethanol
- Sealing film
- Optional: Mixer Mill, such as a Spex Certiprep GenoGrinder 2010
- Optional: Incubator capable of 95°C

Before Starting:

- Set an incubator to 37°C and 70°C.
- Heat Elution Buffer to 70°C.
- Prepare Lysozyme (50 mg/mL) for Step 8.
- Prepare an ice bucket and prechill P2 Buffer on ice.
- Prepare 70% ethanol for use in Step 28. A minimum of 1 mL is required per sample.
- Prepare VHB Buffer according to the "Preparing Reagents" section on Page 4.
- Prepare each 15 mL centrifuge tube with beads from 1 well of E-Z 96 Disruptor Plate C Plus according to the "Preparing Reagents" section on Page 4.
- Optional: for gram-positive bacteria, set an incubator or water bath to 95°C.

1. Filter the water samples using microporous filter paper (0.22 µm or 0.45 µm).

   Note: The volume of water to be filtered depends on the microbial load and turbidity of the water sample. For turbid water samples, it is highly recommended to use prefilter paper to prevent clogging of the microporous filter.

2. After water has filtered through, leave the vacuum on for one additional minute to dry the filter.

3. Turn off the vacuum and remove the filter adapter.

4. Let the filter dry on the adapter for 5 minutes at room temperature.

5. Remove the filter from the adapter, cut the membrane into four pieces, and place the filter in a 15 mL centrifuge tube prefilled with E-Z 96 Disruptor Plate C Plus beads.

   Note: Please see Page 4 for instruction on how to fill a 15 mL centrifuge tube with E-Z 96 Disruptor Plate C Plus beads.

6. Add 1,050 µL SLX-Mlus Buffer.

7. Vortex at maximum speed for 5 minutes to lyse and homogenize samples.

8. Add 20 µL lysozyme (not provided). Briefly vortex the tubes.

9. Incubate 37°C for 20 minutes.

10. Add 350 µL DS Buffer.

11. Vortex at maximum speed for 5 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as a Spex Certiprep GenoGrinder 2010 or Beadmill homogenizer for 15 mL tubes, should be used.

12. Incubate at 70°C for 10 minutes. Briefly vortex the tubes once during incubation.

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

13. Centrifuge at 2,000*g* for 10 minutes at room temperature.

14. Transfer as much supernatant as possible (up to 1 mL) to a new 1.5 mL or 2.0 mL microcentrifuge tube.

15. Add 134 µL prechilled P2 Buffer and 200 µL cHTR Reagent. Vortex to mix thoroughly.

    Note: Prechill P2 Buffer on ice before use. Completely resuspend cHTR Reagent by shaking the bottle before use. If necessary, cut the pipet tip to transfer cHTR Reagent.

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

17. Transfer up to 800 µL supernatant without disturbing the pellet to a 96-well deep-well plate (not provided; must be compatible with the magnetic separation device used).

18. Add 1 volume XP1 Buffer and 20 µL Mag-Bind® Particles RQ.

    Note: Mag-Bind® Particles RQ and XP1 Buffer can be prepared as a mastermix prior to use. Prepare only what is needed.

19. Vortex for 5 minutes at room temperature.

    Note: If constant vortexing is not possible, vortex for 15 seconds every 1-2 minutes for 5 minutes.

20. Place the plate on a magnetic separation device to magnetize the Mag-Bind® Particles RQ. Let sit for 5 minutes or until Mag-Bind® Particles RQ have completely migrated to the well wall.

    Note: If Mag-Bind® Particles RQ at the top of well are not completely migrating, pipet up and down while plate is on magnet to help move Mag-Bind® Particles RQ at top closer to magnetic field.

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

22. Remove the plate containing the Mag-Bind® Particles RQ from the magnetic separation device.

23. Add 500 µL VHB Buffer.

    Note: VHB Buffer must be diluted with 100% ethanol prior to tuse. Please see Page 4 for instructions.

24. Vortex for 30-60 seconds at room temperature.

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

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

27. Remove the plate containing the Mag-Bind® Particles RQ from the magnetic separation device.

28. Add 500 µL 70% ethanol

29. Vortex for 30-60 seconds at room temperature.

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

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

32. Repeat Steps 27-31 for a second 70% ethanol wash step.

33. Leave the plate on the magnetic separation device. Wait 1 minute. Remove any residual liquid with a pipette.

34. Leave the plate on the magnetic separation device for 10 minutes to air dry the Mag-Bind® Particles RQ.

35. Add 50-100 µL Elution Buffer preheated to 70°C.

36. Vortex for 5 minutes at room temperature.

    Note: If constant vortexing is not possible, vortex for 15 seconds every 1-2 minutes for 5 minutes.

37. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles RQ. Let sit at room temperature until the Mag-Bind® Particles RQ are completely cleared from solution.

38. Transfer the cleared supernatant containing purified DNA to a clean 96-well microplate (not provided) and seal with sealing film (not provided).

39. Store the DNA 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 **(800-832-8896)**.

Problem	Cause	Solution
Low Yield or no DNA Yield	Sample stored incorrectly	Sample should be stored at 4°C or -20°C.
	Poor homogenization of sample	Repeat the DNA isolation with a new sample, but be sure to vortex the sample with SLX-Mlus and ceramic beads thoroughly.
	Difficult to lyse bacteria	Follow optional lysozyme step in Water Protocol.
	Incorrect volume XP1 Buffer was added before binding to the beads	Repeat the DNA isolation with a new sample.
	DNA washed off	Use 70% ethanol to wash the Mag-Bind® Particles RQ.
$A_{260/230}$ 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 Mag-Bind® Particles RQ are dry before elution. Wash the Mag-Bind® Particles RQ with extra 70% ethanol.
	70% ethanol contains impurities	Check the absorbance of the ethanol between 250 nm and 300 nm. Do not use ethanol with high absorbance. Traces of impurities may be on the beads after washing and contribute to the absorbance in the final product.
	RNA contamination	Use RNase A (10 mg/mL) at the correct step.
Problems in downstream applications	Too much DNA inhibits PCR reaction	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 beads before elution.

Notes:

For more purification solutions, visit **www.omegabiotek.com**

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

SAMPLE TYPES


Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

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