OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® Universal Metagenomics Kit

M5633-00	5 Preps
M5633-01	50 Preps

April 2017

For research use only.Not intended for diagnostic testing.

Mag-Bind® Universal Metagenomics Kit

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Manual Revision: April 2017

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Introduction and Overview

Environmental metagenomics involves dealing with a nearly infinite number of different combinations of microbiome communities and sample types. Current commercial extraction kits are designed for speed and ease of use, and are not nearly robust enough to consistently extract an adequate yield and representation of DNA from the resident microbiome for downstream analyses.

Omega Bio-tek has partnered with the Extreme Microbiome Project (XMP) to develop the Mag-Bind® Universal Metagenomics Kit for DNA extraction from organisms found in extreme environments that is also compatible for most environmental sample types. This new kit employs a hybrid, self-customizable protocol that covers proper pretreatment for a variety of sample types, a core multi-lysis (mechanical, enzymatic, and chemical) extraction protocol, along with other optional sample type specific steps. This approach provides investigators the ability to use one single kit to optimize and maintain their DNA extraction method for consistent results and analysis.

Omega Bio-tek, Inc. would like to thank the Extreme Microbiome Project (XMP) for their collaboration in co-developing this product.

Kit Contents

Product Number	M5633-00	M5633-01
Purifications	5 Preps	50 Preps
Mag-Bind® Particles RQ	100 µL	1 mL
SLX-Mlus Buffer	3 mL	30 mL
DS Buffer	300 µL	3 mL
ML1 Buffer	8 mL	75 mL
cHTR Reagent	10 mL	100 mL
RBB Buffer	15 mL	140 mL
VHB Buffer	4.4 mL	44 mL
Elution Buffer	1.5 mL	10 mL
Proteinase K Solution	300 µL	3 mL
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Storage and Stability

All of the Mag-Bind® Universal Metagenomics Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles RQ must be stored at 2-8°C. Proteinase K Solution can be stored at room temperature for up to 12 months. For long-term storage, store Proteinase K Solution at 2-8°C. All remaining components should be stored at room temperature. During shipment or storage at cool ambient conditions, precipitates may form in some of the buffers. Dissolve such deposits by warming the solution at 37°C with gentle shaking.

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

Kit	100% Ethanol to be Added
M5633-00	5.6 mL
M5633-01	56 mL

Homogenizer Settings

The following manufacturers' settings have been tested by Omega Bio-tek or provided by end users.

* indicates Omega Bio-tek provided values

Omni Bead Ruptor 24 Elite*: Speed 4, 3 cycles of 30 seconds on/30 seconds dwell

MP Bio FastPrep®: 5K for 30 seconds

Biospec Mini-Beadbeater-1: 1-2 minutes on high

Spex® Sample Prep 2010 Geno/Grinder®*: 2 minutes at 1500 RPM

Spex® Sample Prep 1600 MiniG® Tissue Homogenizer & Cell Lyser*: 2 minutes at 1500 RPM

Mag-Bind® Universal Metagenomics Kit - Liquid Sample Protocol

Optional Steps: It is highly recommended to follow the complete protocol the first time the product is tested. Depending on sample type, the following steps are optional:

Step 4: Mechanical disruption: This step increases the efficiency of bacterial lysis but can cause reduction of DNA fragment size.
Steps 7-11: Proteinase K treatment: This step is for samples that may contain encapsulated bacteria.
Step 14: Heat & Freeze: This step increases the efficiency of spore and bacterial lysis.
Steps 20-23: Phenol/Chloroform: This step provides additional lysis and sample purity.
Step 24: RNase A treatment: This step degrades RNA to prevent co-purification.

Materials and Equipment to be Supplied by User:

- 2 mL screw cap centrifuge tubes
- 1.5 and 2.0 mL microcentrifuge tubes
- Omega Bio-tek Disruptor Tubes (Cat#AC1733) or MP Biomedicals Lysing Matrix A tubes
- Microcentrifuge capable of 14,000 x *g*
- Incubators capable of 37°C, 55°C, 70°C, and 80°C
- -80°C freezer
- Vortexer
- Magnetic Separation Device for 2.0 mL tubes (Cat#MSD-02)
- RNase A (10 mg/mL)
- 100% ethanol
- 80% ethanol
- 10X TE (100 mM Tris-Cl, 10 mM EDTA, pH 7.5)
- Tris-saturated Phenol:Chloroform:Isoamyl Alcohol (25:24:1)
- MetaPolyzme (10 mg/mL, Sigma Aldrich Cat# MAC4L) or lysozyme (50 mg/mL, Worthington Biomedical Cat#LS002881)
- ***Optional:*** Quanta Biosciences Phase Lock Gel Heavy (Quanta Cat#2302830)
- ***Optional:*** PBS, pH 7.5 if using MetaPolyzme. **PBS must be pH 7.5**
- ***Optional:*** Omni International Bead Ruptor 24 or equivalent

Before Starting:

- Prepare VHB Buffer according to the "Preparing Reagents" section on Page 4.
- Set an incubator to 37°C.
- Set an incubator to 55°C.
- Set an incubator to 80°C
- Heat Elution Buffer to 70°C.

Mag-Bind® Universal Metagenomics Kit
Liquid Samples Protocol

1. Add up to 200 µL sample to a 2mL screw cap centrifuge tube (not provided).

2. Centrifuge at 5,000 x *g* for 3 minutes. Remove and discard supernatant

3. Add 200 µL SLX-Mlus Buffer if digesting samples with lysozyme in Step 12 or use 200 µL PBS, pH 7.5 if using Metapolyzyme in Step 12. Vortex at maximum speed to resuspend the sample.

4. Transfer entire sample to a Disruptor Tube or MP Lysing Matrix A tube (not provided). Alternatively, lysing matrix can be added directly to the samples in screw cap tube.

   Note: Cut off the pipet tip to make sample transfer easier.

5. Vortex at maximum speed for 3-5 minutes to lyse and homogenize the samples. For best results, an Omni Bead Ruptor 24 should be used.

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

7. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

8. Incubate at 55°C for 30 minutes.

9. Incubate at 80°C for 10 minutes.

   Note: This step deactivates Proteinase K and DNases in the sample.

10. Let the sample cool to room temperature.

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

12. Add 25 µL MetaPolyzme (10 mg/mL) or lysozyme (50 mg/mL) (not provided). Vortex to mix thoroughly.

    Note: MetaPolyzme cannot be used in Tris-based buffers. PBS must be pH 7.5.

13. Incubate at 37°C for 4-24 hours.

14. Incubate at 80°C for 1-2 minutes then immediately move to -80°C freezer for 5 minutes. Repeat as needed, performing at least 2 cycles. Let the sample cool to room temperature before proceeding.

15. Add 25 µL 10X TE (not provided), 13 µL DS Buffer, and 25 µL Proteinase K Solution. Vortex to mix thoroughly.

16. Incubate at 55°C for 30 minutes.

17. Add 500 µL ML1 Buffer. Vortex to mix thoroughly.

    Note: Sample may become cloudy or a precipitate may form during and/or in the steps following the addition of ML1 Buffer. This is normal and will clear after RBB Buffer is added in Step 25 below.

18. Incubate at 55°C for 15 minutes.

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

20. Add an equal volume of Phenol:Chloroform:Isoamyl Alcohol (25:24:1). Vortex to mix thoroughly.

21. Let sit at room temperature for 5 minutes.

Optional: Samples can be transferred to Quanta Biosciences Phase Lock Gel Heavy (not provided) for Steps 22-23 for efficient separation of organic/aqueous phases.

22. Centrifuge at 10,000 x *g* for 10 minutes.

23. Transfer no more than 500 µL top aqueous phase to a new 2 mL microcentifuge tube (not provided).

24. Add 1 µL RNase A (not provided) and incubate at 37°C for 5 minutes.

25. Add 2 volumes RBB Buffer.

26. Add 1/4 volume 100% ethanol, and 15 µL Mag-Bind® Particles RQ. Vortex to mix thoroughly or pipet up and down 20 times.

    Example: For 500 µL aqueous phase transferred, add 1000 µL RBB Buffer, 375 µL 100% ethanol, and 15 µL Mag-Bind® Particles RQ.

27. Let sit at room temperature for 10 minutes.

    Note: For optimal yields, constantly shake or rotate tubes during incubation. If incubating without agitation/mixing, vortex the samples at least twice during incubation.

28. Place the tube 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.

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

30. Remove the tube containing the Mag-Bind® Particles RQ from the Magnetic Separation Device.

31. Add 600 µL VHB Buffer. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

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

32. Let sit at room temperature for 2 minutes.

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

35. Remove the tube containing the Mag-Bind® Particles RQ from the Magnetic Separation Device.

36. Repeat Steps 31-35 for a second VHB Buffer wash step.

37. Add 600 µL 80% ethanol. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

38. Let sit at room temperature for 2 minutes.

39. Place the tube 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.

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

41. Remove the tube containing the Mag-Bind® Particles RQ from the Magnetic Separation Device.

42. Repeat Steps 37-41 for a second ethanol wash step.

43. Leave the tube on the Magnetic Separation Device. Tap the tubes/magnet to collect extra buffer at the bottom of the tube and remove any remaining liquid with a pipette.

44. Dry the Mag-Bind Particles RQ on the magnet at room temperature for 10 minutes.

45. Remove the tube from the Magnetic Separation Device.

46. Add 25-50 µL Elution Buffer heated to 70°C. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

47. Let sit at room temperature for 5 minutes.

48. Place the tube 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.

49. Transfer the cleared supernatant containing purified DNA to a new 1.5 mL microcentrifuge tube (not provided). Store DNA at -20°C.

Mag-Bind® Universal Metagenomics Kit - **Inhibitor Rich or Solid Sample Protocol**

Optional Steps: It is highly recommended to follow the complete protocol the first time the product is tested. Depending on sample type, the following steps are optional:

Step 3: Mechanical disruption: This step increases the efficiency of bacterial lysis but can cause reduction of DNA fragment size.
Steps 5-8: Proteinase K treatment: This step is for samples that may contain encapsulated bacteria.
Step 12: Heat & Freeze: This step increases the efficiency of spore and bacterial lysis.
Steps 20-23: Phenol/Chloroform: This step provides additional lysis and sample purity.
Step 24: RNase A treatment: This step degrades RNA to prevent co-purification.
Step 25-28: cHTR Reagent: This step uses cHTR Reagent to remove inhibitors/color from samples.

Materials and Equipment to be Supplied by User:

- 1.5 mL microcentrifuge tubes
- 15 mL centrifuge tubes
- Omega Bio-tek Disruptor Tubes (Cat#AC1733) or MP Biomedicals Lysing Matrix A tubes
- Microcentrifuge capable of 14,000 x *g*
- Centrifuge capable of 4,000 x *g* with adaptors for 15 mL centrifuge tubes
- Incubators capable of 37°C, 55°C, 70°C, and 80°C
- -80°C freezer
- Vortexer
- Magnetic Separation Device for 15 mL tubes (Cat#MSD-02)
- RNase A (10 mg/mL)
- 100% ethanol
- 80% ethanol
- 10X TE (100 mM Tris-Cl, 10 mM EDTA, pH 7.5)
- Tris-saturated Phenol:Chloroform:Isoamyl Alcohol (25:24:1)
- MetaPolyzme (10 mg/mL, Sigma Aldrich Cat# MAC4L) or lysozyme (50 mg/mL, Worthington Biomedical Cat#LS002881)
- ***Optional:*** Magnetic Separation Device for 1.5/2.0 mL microcentrifuge tubes (Cat#MSD-02)
- ***Optional:*** PBS, pH 7.5 if using MetaPolyzme. **PBS must be pH 7.5**
- ***Optional:*** Omni International Bead Ruptor 24 or equivalent

Mag-Bind® Universal Metagenomics Kit
Inhibitor Rich or Solid Sample Protocol

Before Starting:

- Prepare VHB Buffer according to the "Preparing Reagents" section on Page 4.
- Set an incubator to 37°C.
- Set an incubator to 55°C.
- Set an incubator to 80°C.
- Heat Elution Buffer to 70°C.

1. Weigh 200-250 mg soil or solid sample into Disruptor Tube or MP Bio Lysing Matrix A tube (not provided).

Important: If using lysozyme in Step 10 below, proceed to Step 2. If using Metapolyzme in Step 10, perform the following steps first and proceed to Step 3.

- Add 1 mL PBS (pH 7.5) and vortex.
- Centrifuge at >10,000 x *g* for 5 minutes.
- Discard the supernatant.
- Resuspend the pellet in 500 µL PBS.
- Proceed to Step 3 below. Do not add SLX-Mlus Buffer.

2. Add 500 µL SLX-Mlus Buffer if digesting with lysozyme in Step 10.

3. Vortex at maximum speed for 3-5 minutes to lyse samples. For best results, an Omni International Bead Ruptor should be used.

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

5. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

6. Incubate at 55°C for 30 minutes.

7. Incubate at 80°C for 10 minutes.

   Note: This step deactivates Proteinase K and DNases in the sample.

8. Let the sample cool to room temperature.

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

10. Add 25 µL MetaPolyzme (10 mg/mL) or lysozyme (50 mg/mL) (not provided). Vortex to mix thoroughly.

    Note: MetaPolyzme cannot be used in Tris-based buffers. PBS must be pH 7.5.

11. Incubate at 37°C for 4-24 hours.

12. Incubate at 80°C for 1-2 minutes then immediately move to -80°C freezer for 5 minutes. Repeat as needed, performing at least 2 cycles. Let the sample cool to room temperature before proceeding.

13. Add 62.5 µL 10X TE (not provided), 32.5 µL DS Buffer, and 25 µL Proteinase K Solution. Vortex to mix thoroughly.

14. Incubate at 55°C for 30 minutes.

15. Vortex for 15-30 seconds.

16. Transfer contents to a 15 mL centrifuge tube (not provided) by inverting the Disruptor Tube into the 15 mL centrifuge tube. Tap to transfer as much as possible. Save the Disruptor Tube for use in the next step.

17. Add 625 µL ML1 Buffer to the empty Disruptor Tube. Vortex 15-30 seconds. Transfer as much as possible to 15mL centrifuge tube from Step 16.

    Note: Sample may become cloudy or a precipitate may form during and/or in the steps following the addition of ML1 Buffer. This is normal and will clear after RBB Buffer is added in Step 29 below.

18. Repeat Step 17 to transfer as much remaining soil to the 15mL centrifuge tube.

19. Incubate at 55°C for 15 minutes.

20. Add an equal volume of Phenol:Chloroform:Isoamyl Alcohol (25:24:1). Vortex to mix thoroughly.

21. Let sit at room temperature for 5 minutes.

22. Centrifuge at 4,000 x *g* for 10 minutes.

23. Transfer no more than 1.2 mL top aqueous phase to a new 15 mL centrifuge tube.

24. Add 1 µL RNase A (not provided) and incubate at 37°C for 5 minutes.

25. Add 1 volume cHTR Reagent. Vortex briefly.

26. Let sit at room temperature for 2 minutes.

27. Centrifuge at 4,000 x *g* for 5 minutes.

28. Transfer supernatant to new 15mL centrifuge tube.

Optional: If lysate is still dark in color (light yellow is acceptable), repeat Steps 25-28 with 0.5 volumes cHTR Reagent.

29. Add 2 volumes RBB Buffer,

30. Add 1/4 volume 100% ethanol and 15 µL Mag-Bind® Particles RQ to each sample. Vortex to mix thoroughly or pipette up and down 20 times.

    Example: For 500 µL aqueous phase transferred, add 1000 µL RBB Buffer and 375 µL 100% ethanol, and 15 µL Mag Bind Particles RQ.

31. Let sit at room temperature for 10 minutes.

    Note: For optimal yields, constantly shake or rotate tubes during incubation. If incubating without agitation/mixing, vortex the samples at least twice during incubation.

32. Place the tube 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.

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

34. Remove the tube containing the Mag-Bind® Particles RQ from the Magnetic Separation Device.

35. Add 600 µL VHB Buffer. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

    Note: VHB Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions. Sample can be transferred to a 1.5 mL microcentrifuge tube at this step.

36. Let sit at room temperature for 2 minutes.

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

39. Remove the tube containing the Mag-Bind® Particles RQ from the Magnetic Separation Device.

40. Repeat Steps 35-39 once for a second VHB Wash step.

41. Add 600 µL 80% ethanol. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

42. Let sit at room temperature for 2 minutes.

43. Place the tube 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.

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

45. Remove the tube containing the Mag-Bind® Particles RQ from the Magnetic Separation Device.

46. Repeat Steps 41-45 for a second ethanol wash step.

47. Leave the tube on the Magnetic Separation Device. Tap the tube/magnet to collect extra buffer at the bottom of the tube and remove with a pipette.

48. Dry the Mag-Bind Particles RQ on the magnet at room temperature for 10 minutes.

49. Remove the tube from the Magnetic Separation Device.

50. Add 25-50 µL Elution Buffer heated to 70°C. Resuspend the Mag-Bind® Particles RQ by vortexing or pipetting up and down 20 times.

51. Let sit at room temperature for 5 minutes.

52. Place the tube 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.

53. Transfer the cleared supernatant containing purified DNA to a new 1.5 mL microcentrifuge tube (not provided). Store 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 **1-800-832-8896**.

Problem	Cause	Solution
A_{260}/A_{230} ratio is low	Salt contamination	• Repeat the DNA isolation with a new sample. • Extend the incubation time with VHB Buffer. • Wash the Mag-Bind® Particles RQ with an increased volume of 80% ethanol and add additional third wash.
A_{260}/A_{280} ratio is high	RNA contamination	The protocol has an optional step to remove RNA. If desired, add 1 µL RNase A (10 mg/mL) and incubate at 37°C for 5 minutes.
Low DNA Yield or no DNA Yield	Poor homogenization of sample	Repeat the DNA isolation with a new sample, be sure to mix the sample with SLX-Mlus Buffer thoroughly. Use a commercial homogenizer if possible.
	DNA washed off	Make sure VHB Buffer is mixed with 100% ethanol.
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.
Problems in downstream applications	Inhibitory substance in the eluted DNA.	Check the A_{260}/A_{230} ratio. Dilute the elute to 1:50 if necessary.

Ordering Information

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

Product	Part Number
Disruptor Tubes (5/pk)	AC1733-05
Disruptor Tubes (50/pk)	AC1733-50
cHTR Reagent (25 mL)	CHTR-50
Magnetic Separation Device (1.5 mL / 2 mL /15 mL centrifuge tubes)	MSD-02
1.5 mL DNase/RNase-free Microcentrifuge Tubes (500/pk, 10 pk/cs)	SS1-1210-00
2.0 mL DNase/RNase-free Microcentrifuge Tubes (500/pk, 10 pk/cs)	SS1-1310-00
RNase A (5 mL)	AC118
Proteinase K (>600 mAU/mL, Solution; 2 mL)	AC115
Proteinase K (>600 mAU/mL, Solution; 10 mL)	AC116