omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

Mag-Bind® Stool DNA 96 Kit

M4016-00	1 x 96 preps
M4016-01	4 x 96 preps

Manual Date: June 2021
Manual Revision: v7.0

For Research Use Only

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Introduction

The Mag-Bind® Stool DNA 96 Kit is designed for rapid and reliable isolation of high quality host as well as pathogenic genomic DNA from stool samples. Up to 300 µL stool samples can be processed in less than 45 minutes. The uniquely formulated cHTR reagent eliminates PCR inhibiting compounds such as humic acids, lipids, etc. commonly found in stool samples. The Mag-Bind® technology is ideally suited for automated liquid handlers. The extraction system allows for autmation after sample lysis via Hamilton Microlab® STAR™, Thermo Fisher Scientific's KingFisher® Flex, Applied Biosystem's MagMAX® 96, Qiagen BioSprint® 96 and other liquid handling platforms.

The kit includes our 96-well disruptor plates which are prefilled with ceramic beads. The protocol involves no organic extractions, reducing both plastic waste and hands on time to allow parallel processing of multiple samples. Two protocols are available depending on the amount of inhibition and downstream sensitivity. It is recommended to begin with the standard protocol and to use the inhibitor-rich sample protocol if issues occur. Purified DNA is inhibitor free and is suitable for various downstream applications such as PCR, restriction digestion and NGS.

If using the Mag-Bind® Stool DNA 96 Kit for the first time, please read this booklet to become familiar with the procedure. Stool sample is homogenized and then treated in a specially formulated buffer. Humic acid, proteins, polysaccharides, and other contaminants are subsequently precipitated. Binding conditions are then adjusted and the DNA is bound to magnetic beads. Four 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.

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:

June 2021
- Mag-Bind® Particles CND has been replaced by Mag-Bind® Particles CH.
- Inhibitor-Rich Sample Protocol has been modified to include optional premix/ mastermix SP2/cHTR. Incubation on ice step has been removed.

March 2019
- XP2 Buffer has been renamed XP2 Binding Buffer and SPM Wash Buffer has been renamed SPM Buffer. These are name change only. The formulations have not changed.

June 2018
- SP2 Buffer and XP2 Buffer volumes supplied with the 4x96 prep kit (M4016-01) have been increased.

Kit Contents

Product Number	M4016-00	M4016-01
Purifications	1 x 96 preps	4 x 96 preps
E-Z 96 Disruptor Plate C with Caps	1	4
SLX-Mlus Buffer	60 mL	240 mL
DS Buffer	5 mL	45 mL
SP2 Buffer	12 mL	45 mL
XP2 Binding Buffer	60 mL	250 mL
VHB Buffer	22 mL	88 mL
SPM Buffer	30 mL	4 x 30 mL
Elution Buffer	30 mL	60 mL
Proteinase K Solution	2.2 mL	8.8 mL
RNase A	220 µL	880 µL
Mag-Bind® Particles CH	2.2 mL	8.8 mL
cHTR Reagent	12 mL	45 mL
User Manual	✓	✓

Storage and Stability

All of the Mag-Bind® Stool DNA 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Mag-Bind® Particles CH, RNase A, and cHTR Reagent 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 in cool ambient conditions, precipitates may form in some of the buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

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

Kit	100% Ethanol to be Added
M4016-00	70 mL
M4016-01	70 mL per bottle

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

Kit	100% Ethanol to be Added
M4016-00	28 mL
M4016-01	112 mL

Mag-Bind® Stool DNA 96 Kit - Standard 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:

- Refrigerated centrifuge capable of at least 4,000*g* and 4°C
- Magnetic Separation Device (Recommended Cat# Alpaqua® 96S A001322)
- Incubator capable of 70°C
- 96-well plates with a capacity of 1200 µL (Abgene AB-1127) and compatible with the Magnetic Separation Device
- 96-well microplates for DNA storage
- Vortexer
- 100% ethanol
- Mixer mill, such as a SPEX CertiPrep Geno/Grinder® 2010 or Omni's Bead Ruptor 96

Before Starting:

- Prepare DNA Wash Buffer and VHB Buffer according to the "Preparing Reagents" section on Page 4.
- Set an incubator to 70°C.
- Heat Elution Buffer to 70°C.
- Shake or vortex the Mag-Bind® Particles CH to fully resuspend the particles before use.

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

2. Add 300 µL stool sample.

3. Add 300 µL SLX-Mlus Buffer and 20 µL Proteinase K. Seal the plate with the caps removed in Step 1.

4. Vortex at maximum speed for 3-5 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Omni International's Bead Ruptor 96, should be used.

   Note: Depending on the sample amount and type, the amount of SLX-Mlus Buffer may need to be adjusted so that 300 µL can be recovered after Step 6.

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

6. Centrifuge at 4,000-6,000*g* for 10 minutes at room temperature.

7. Transfer 300 µL supernatant to a 96-well plate compatible with the Magnetic Separation Device used.

8. Add 600 µL XP2 Binding Buffer and 20 µL Mag-Bind® Particles CH.

   Note: Mag-Bind® Particles CH and XP2 Binding Buffer can be prepared as a mastermix prior to use. Prepare only what is needed.

9. Vortex for 5 minutes at room temperature.

   Note: If constant vortexing for 5 minutes is not possible, vortex for 30 seconds every minute for 5 minutes.

10. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

11. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

12. Remove the plate containing the Mag-Bind® Particles CH from the Magnetic Separation Device.

13. Add 400 µL VHB Buffer. Resuspend the Mag-Bind® Particles CH by vortexing or pipetting up and down 20 times.

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

14. Let sit for 2 minutes at room temperature.

15. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

17. Remove the plate containing the Mag-Bind® Particles CH from the Magnetic Separation Device.

18. Add 400 µL SPM Buffer. Resuspend the Mag-Bind® Particles CH by vortexing or pipetting up and down 20 times.

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

19. Let sit for 2 minutes at room temperature.

20. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

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

22. Repeat Steps 17-21 for a second SPM Buffer wash step.

23. Leave the plate on the Magnetic Separation Device for 5-10 minutes to air dry the Mag-Bind® Particles CH. Remove any residual liquid with a pipettor.

24. Add 50-100 µL Elution Buffer. Resuspend the Mag-Bind® Particles CH by vortexing or pipetting up and down 20 times.

25. Let sit for 5 minutes at room temperature.

26. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

27. Transfer the cleared supernatant containing purified DNA to a clean plate. Store the DNA at -20°C.

Mag-Bind® Stool DNA 96 Kit - Inhibitor-Rich Protocol

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

Materials and Equipment to be Supplied by User:

- Refrigerated centrifuge capable of at least 4,000*g* and 4°C
- Magnetic Separation Device (Recommended Cat# Alpaqua® 96S A001322)
- Incubator capable of 70°C
- 96-well plates with a capacity of 1200 µL (Abgene AB-1127) and compatible with the Magnetic Separation Device
- 96-well microplates for DNA storage
- Vortexer
- Sealing film
- 100% ethanol
- Mixer mill, such as a SPEX CertiPrep Geno/Grinder® 2010 or Omni's Bead Ruptor 96

Before Starting:

- Prepare DNA Wash Buffer and VHB Buffer according to the "Preparing Reagents" section on Page 4.
- Set an incubator to 70°C.
- Heat Elution Buffer to 70°C.

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

2. Add 300 µL stool sample.

3. Add 300 µL SLX-Mlus Buffer and 20 µL Proteinase K. Seal the plate with the caps removed in Step 1.

4. Vortex at maximum speed for 3-5 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Omni International's Bead Ruptor 96, should be used.

   Note: Depending on the sample amount and type, the amount of SLX-Mlus Buffer may need to be adjusted so that 300 µL can be recovered after Step 9.

5. Remove and save the caps.

6. Add 30 µL DS Buffer and 2 µL RNase A. Seal the plate with the caps.

7. Vortex to mix thoroughly.

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

9. Centrifuge at 4,000-6,000*g* for 10 minutes at room temperature.

10. Transfer 300 µL supernatant to a new 96-well plate (not provided).

11. Add 100 µL SP2 Buffer and 100 µL cHTR Reagent. Seal the plate with sealing film. Vortex to mix thoroughly.

    Note: Completely resuspend the cHTR Reagent by shaking the bottle before use. It may be necessary to cut the end of the pipette tip to aspirate and dispense cHTR Reagent.

    Note: SP2 Buffer and cHTR Reagent can be prepared as a mastermix prior to use. This mastermix can be stored at room temperature for up to 1 month.

12. Let sit at room temperature for 5 minutes.

13. Centrifuge at 4,000-6,000*g* for 10 minutes at room temperature.

14. Transfer 300 µL supernatant to a new 96-well plate compatible with the Magnetic Separation Device used.

15. Add 600 µL XP2 Binding Buffer and 20 µL Mag-Bind® Particles CH.

    Note: Mag-Bind® Particles CH and XP2 Binding Buffer can be prepared as a mastermix prior to use. Prepare only what is needed.

16. Vortex for 5 minutes at room temperature.

    Note: If constant vortexing for 5 minutes is not possible, vortex for 30 seconds every minute for 5 minutes.

17. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

18. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

19. Remove the plate containing the Mag-Bind® Particles CH from the Magnetic Separation Device.

20. Add 400 µL VHB Buffer. Resuspend the Mag-Bind® Particles CH by vortexing or pipetting up and down 20 times.

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

21. Let sit for 5 minutes at room temperature.

22. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

23. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

24. Remove the plate containing the Mag-Bind® Particles CH from the Magnetic Separation Device.

25. Add 400 µL SPM Buffer. Resuspend the Mag-Bind® Particles CH by vortexing or pipetting up and down 20 times.

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

26. Let sit for 2 minutes at room temperature.

27. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

28. Aspirate and discard the cleared supernatant. Do not disturb the Mag-Bind® Particles CH.

29. Repeat Steps 24-28 for a second SPM Buffer wash step.

30. Leave the plate on the Magnetic Separation Device for 5-10 minutes to air dry the Mag-Bind® Particles CH. Remove any residual liquid with a pipettor.

31. Add 50-100 µL Elution Buffer. Resuspend the Mag-Bind® Particles CH by vortexing or pipetting up and down 20 times.

32. Let sit for 5 minutes at room temperature.

33. Place the 96-well plate on the Magnetic Separation Device to magnetize the Mag-Bind® Particles CH. Let sit at room temperature until the Mag-Bind® Particles CH are completely cleared from solution.

34. Transfer the cleared supernatant containing purified DNA to a clean plate. Store the DNA at -20°C.

Mag-Bind® Stool DNA 96 Kit - KingFisher™ Flex, KingFisher™ 96, Applied Biosystems MagMAX™, Qiagen Biosprint Protocol

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

Materials and Equipment to be Supplied by User:

- Refrigerated centrifuge capable of at least 4,000*g* and 4°C
- Thermo KingFisher™ Flex/Applied Biosystems MagMAX™/Qiagen Biosprint
- Incubator capable of 70°C
- KingFisher™ Deep Well plates
- KingFisher™ 96 KF microplate (200 µL) for DNA storage
- Vortexer
- Ice bucket
- 100% ethanol
- Nuclease-free water
- Mixer mill, such as a SPEX CertiPrep Geno/Grinder® 2010 or Omni International's Bead Ruptor 96

Before Starting:

- Set an incubator to 70°C

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

2. Add 300 µL stool sample.

3. Add 300 µL SLX-Mlus Buffer and 20 µL Proteinase K. Seal the plate with the caps removed in Step 1.

4. Vortex at maximum speed for 3-5 minutes to lyse and homogenize samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Omni International's Bead Ruptor 96, should be used.

   Note: Depending on the sample amount and type, the amount of SLX-Mlus Buffer may need to be adjusted so that 300 µL can be recovered after Step 7.

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

6. Centrifuge at 4,000-6,000*g* for 10 minutes at room temperature.

7. Transfer 300 µL supernatant to a KingFisher™ 96 Deep Well plate.

8. Add 600 µL XP2 Binding Buffer and 20 µL Mag-Bind® Particles CH. ***This is the Lysate Plate.***

   Note: Mag-Bind® Particles CH and XP2 Binding Buffer can be prepared as a mastermix prior to use. Prepare only what is needed.

9. Prepare the remaining plates as follows. The Lysate Plate was prepared in Steps 1-8.

Plate Type	Name	Contents	Volume
Deep Well	Lysate	Sample XP2 Binding Buffer Mag-Bind® Particles CH	300 µL 600 µL 20 µL
Deep Well	VHB	VHB Buffer	400 µL
Deep Well	SPM 1	SPM Buffer	400 µL
Deep Well	SPM 2	SPM Buffer	400 µL
Deep Well	Elution	Elution Buffer	500 µL
Microplate	Tip Pick Up	Magnetic Tip Comb	100 µL
Empty	Empty	Empty	
Empty	Empty	Empty	

10. Press start on the instrument protocol and load the plates according to the prompts.

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 the sample with cHTR Reagent thoroughly. Add 100 µL to cleared supernatant. Mix by vortexing. Let sit for two minutes. Centrifuge at 4,000*g* for 10 minutes and transfer cleared supernatant to next step. Do not reuse SP2 Buffer.
	Salt contamination	• Repeat the DNA isolation with a new sample. • Extend the incubation time with VHB Buffer.
A_{260}/A_{280} ratio is high	RNA contamination	Be sure to treat the sample with RNase A according to the protocol.
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 and SPM Buffer are mixed with 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.
	Ethanol residue in the elute	Extend the dry time of the Mag-Bind® Particles CH to 15 minutes before elution.

Notes:

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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