Mag-Bind® Stool DNA Kit

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Introduction

The Mag-Bind® Stool DNA Kit allows rapid and reliable isolation of high-quality total DNA from fresh and frozen stool samples. Up to 200 mg of stool samples can be processed in less than 60 minutes. The system combines the reversible nucleic acid binding properties of Mag-Bind® particles with the efficiency of HTR Reagents to eliminate humic acid, polysaccharides, phenolic compounds, and enzyme inhibitors from stool 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.

Overview

Stool samples typically contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions. Mag-Bind® Stool DNA Kit uses a unique HTR Reagent and SP2 Buffer that can remove inhibitory substances from stool samples.

If using the Mag-Bind® Stool DNA Kit for the first time, please read this booklet to become familiar with the procedures. Frozen or fresh stool samples are homogenized and then lysed in a specially formulated buffer containing detergent. Proteins, polysaccharides, and cellular debris are subsequently precipitated with SP2 Buffer after a heat-freeze step. Contaminants are further removed by HTR reagent by a quick centrifuge step. Binding conditions are then adjusted and the DNA is will be selectively bind to the surface of Mag-Bind® Particles. Two rapid wash steps remove trace contaminants, and pure DNA is eluted in DNA Elution Buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

New in this Edition:

November 2019
This manual has been edited for content and redesigned to enhance user readability.

Kit Contents

Product Number	M4015-00	M4015-01	M4015-02
Purifications	5 preps	50 preps	200 preps
Mag-Bind® Particle	120 µL	1.2 mL	4.2 mL
Buffer SLX	10 mL	90 mL	2 x160 mL
Buffer SP2	3 mL	30 mL	120 mL
HTR Reagent	1.2 mL	12 mL	50 mL
Buffer VHB	2 mL	22 mL	88 mL
Binding Buffer	4 mL	30 mL	100 mL
SPM Wash Buffer	3 mL	30 mL	2 x 60 mL
OB Protease	150 µL	1.2 mL	4 x 1.2 mL
Glass Beads	1.2 g	12 g	45 g
Elution Buffer	2 mL	15 mL	60 mL
User Manual	✓	✓	✓

Storage and Stability

All of the Mag-Bind® Stool DNA Kit components are guaranteed for at least 24 months from the date of purchase when stored as follow. OB Protease should be stored at 15-25°C. Mag-Bind® Particles Solution should be stored at 4°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 Buffer SLX. 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
M4015-00	7 mL
M4015-01	70 mL
M4015-02	140 mL per bottle

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

Kit	100% Ethanol to be Added
M4015-00	3 mL
M4015-01	28 mL
M4015-02	112 mL

Mag-Bind® Stool DNA Kit - Human DNA Detection Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000 x g
- Nuclease-free 1.5 mL and 2 mL microfuge tubes
- Water bath equilibrated to 65 °C
- 100% ethanol
- RNase A stock solution at 20 mg/mL(optional)

1. Weigh up to 200 mg stool sample in a 2 mL centrifuge tube (not supplied) and place the tube on ice. Add 1.2 mL Buffer SLX and vortex at maximum speed for 1 minute or until the stool sample is throughly homogenized.

   Note: If the sample is liquid, pipet 200 µL of sample into the centrifuge tube. Cut the end of the pipet tip to make pipetting easier. If the sample is frozen, use a spatula to scrape the sample into the tube. Do not thaw the frozen sample until the Buffer SLX is added into the tube.

2. Add 400 µL of Buffer SP2 and mix throughly by vortexing for 10 seconds. Incubate on ice for 5 minutes.

3. Centrifuge at full speed (>14,000 x g) for 3 minutes at room temperature.

4. Transfer 1 mL of cleared supernatant to a new 2 mL tube and add 200 µL of HTR Reagent. Mix the sample throughly by voretxing for 10 seconds.

   Important: HTR reagent must be throughly suspended before being dispense from bottle. Tip: Use 1 mL pipettor and cut off the end of 1ml tip to make it easier for pipetting the HTR reagent.

5. Incubate at room temperature for 2 minutes.

6. Centrifuge at full speed (>13,000 x g) for 2 minutes to pellet the inhibitors absorb to HTR Reagent.

7. Transfer 600 µL of supernatant into a new 2.0 ml centrifuge tube (not supplied). Add 20 µL of OB Protease(20mg/ml) and mix throughly by vorexting.

8. Add 300 µL of Binding Buffer and 20 µL Mag-Bind® Particle. Mix by vortexing for 30 seconds.

9. Place the tube or plate on a Magnetic Separation Device to magnetize the Mag-Bind® Particles. Completely aspirate the cleared supernatant by pipetting.

10. Remove the tube or plate containing the Mag-Bind® Particles from the Magnetic Separation Device. Add 700 µL of Buffer VHB/ethanol for each sample.

11. Resuspend Mag-Bind® Particles by vortexing or pipetting. Incubate 3 minutes at room temperature. Mix by vortexing or pipetting few times during incubation.

12. Place the tube or plate onto a Magnetic Separation Device to magnetize the Mag-Bind® Particles. Completely aspirate the cleared supernatant by pipetting.

13. Remove the tube or plate containing the Mag-Bind® Particles from the Magnetic Separation Device. Add 700 µL of SPM Wash Buffer for each sample.

14. Resuspend Mag-Bind® Particles by vortexing or pipetting. Incubate 3 minutes at room temperature. Mix by vortexing or pipetting few times during incubation.

    Note: It is critical to wash the Mag-Bind® Particles by breaking up the Mag-Bind® Particles.

15. Completely aspirate the cleared supernatant by pipetting.

16. Remove the tube or plate containing the Mag-Bind® Particles from the Magnetic Separation Device. Add 700 µL of SPM Wash Buffer for each sample.

17. Resuspend Mag-Bind® Particles by vortexing or pipetting up and down.

18. Place the tube or plate onto a Magnetic Separation Device to magnetize the Mag-Bind® Particles.

19. Completely aspirate the cleared supernatant by pipetting.

20. Leave the tube or plate to air dry on the Magnetic Separation Device for 5-10 minutes. Remove any residual liquid with a pipettor.

    Note: Briefly centrifuge the tubes to bring down any residual liquid remove by pipetting off.

21. Remove the tube or plate from Magnetic Separation Device. Add 50-100 µL Elution Buffer or water to elute DNA from Mag-Bind® Particles.

22. Resuspend the Mag-Bind® Particles by vortexing 3 minutes or pipetting up and down 50 times. Let sit at room temperature for 5-10 minutes.

23. Place the tube or plate onto the Magnetic Separation Device to magnetize the Mag-Bind® Particles. Let sit at room temperature until the Mag-Bind® Particles are completely cleared from solution.

24. Transfer the cleared supernatant containing purified DNA to a new 1.5mL tube or clean plate. Store the DNA at -20°C.

    Note: Incubation at 70 °C rather than at room temperature will give a modest increase in DNA yield per elution. Alternatively the second elution may be performed using the first eluate.

Mag-Bind® Stool DNA Kit - Pathogen Detection Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000 x g
- Nuclease-free 1.5 mL and 2 mL microfuge tubes
- Water bath equilibrated to 65 °C
- 100% ethanol
- RNase A stock solution at 20 mg/mL(optional)

1. Weigh up to 50-100 mg of stool sample in a 2 mL centrifuge tube containing 200 mg of glass beads and place the tube on ice.

   Note: If the sample is liquid, pipet 200µL of sample into the centrifuge tube. Cut the end of the pipet tip to make pipetting easier. If the sample is frozen, use a spatula to scrape the sample into the tube. Do not thaw the frozen sample until the Buffer SLX/ OB Protease is added into the tube.

2. Add 600 µL Buffer SLX followed by then adding 20 µL of OB Protease. Vortex at maxi speed for 3 minutes.

3. Incubate at 70 °C for 10 min (13 min if frozen). Mix sample twice during incubation by vortexing the tube.

   Optional: For isolation of DNA from gram positive bacteria, do a second incubation at 95°C for 5 minutes.

4. Add 200 µL of Buffer SP2. Mix the sample throughly by voretxing the tube for 30 seconds. Incubate the sample on ice for 5 minutes.

5. Centrifuge at full speed (13,000-20,000 x g) in a microcentrifuge for 5 minutes to pellet the stool particles.

6. Carefully aspirate supernatant to a new 1.5 mL microfuge tube (not supplied), making sure not to disturb the pellet or transfer any debris.

7. Add 200 µL of HTR Reagent. Mix the sample by vortexing the tube for 10 seconds. Incubate at room temperature for 2 minutes.

   Important: HTR Reagent must be throughly suspended before being dispense from bottle.

   Tip: Use 1 mL pipettor and cut off the end of 1 mL tip to make it easier for pipetting the HTR Reagent. Completely resuspend the HTR Reagent by shaking the bottle before use.

8. Centrifuge at full speed (>13,000 x g) for 2 minutes to pellet the inhibitors absorb to HTR Reagent.

9. Transfer 600 µL supernatant to a new 2.0 mL tube. Add 300 µL of Bindin Buffer and 20 µL Mag-Bind® Particles. Mix by vortexing for 30 seconds.

10. Place the tube or plate on a Magnetic Separation Device to magnetize the Mag-Bind® Particles. Completely aspirate the cleared supernatant by pipetting.

11. Remove the tube or plate containing the Mag-Bind® Particles from the Magnetic Separation Device. Add 700 µL of Buffer VHB/ethanol for each sample.

12. Resuspend Mag-Bind® Particles by vortexing or pipetting. Incubate 3 minutes at room temperature. Mix by vortexing or pipetting few times during incubation.

    Note: It is critical to wash the Mag-Bind® Particles by breaking up the Mag-Bind® Particles.

13. Remove the tube or plate containing the Mag-Bind® Particles from the Magnetic Separation Device. Add 700 µL of SPM Wash Buffer for each sample.

14. Resuspend Mag-Bind® Particles by vortexing or pipetting. Incubate 3 minutes at room temperature. Mix by vortexing or pipetting few times during incubation.

    Note: It is critical to wash the Mag-Bind® Particles by breaking up the Mag-Bind® Particles.

15. Completely aspirate the cleared supernatant by pipetting.

16. Remove the tube or plate containing the Mag-Bind® Particles from the Magnetic Separation Device. Add 700 µL of SPM Wash Buffer for each sample.

17. Resuspend Mag-Bind® Particles by vortexing or pipetting.

18. Place the tube or plate on a Magnetic Separation Device to magnetize the Mag-Bind® Particles.

19. Completely aspirate the cleared supernatant by pipetting.

20. Leave the tube or plate to air dry on the Magnetic Separation Device for 5-10 minutes. Remove any residual liquid with a pipettor.

    Note: Briefly centrifuge the tubes to bring down any residual liquid remove by pipetting off.

21. Remove the tube or plate from Magnetic Separation Device. Add 50-100 µL Elution Buffer or water to elute DNA from Mag-Bind® Particles.

22. Resuspend the Mag-Bind® Particles by vortexing 3 minutes or pipetting up and down 50 times. Let sit at room temperature for 5-10 minutes.

23. Place the tube or plate onto the Magnetic Separation Device to magnetize the Mag-Bind® Particles. Let sit at room temperature until the Mag-Bind® Particles are completely cleared from solution.

24. Transfer the cleared supernatant containing purified DNA to a new 1.5mL tube or clean plate. Store the DNA at -20°C.

    Note: Incubation at 70 °C rather than at room temperature will give a modest increase in DNA yield per elution. Alternatively the second elution may be performed using the first eluate.

Tip: For maximum PCR robustness, it is recommended to add BSA to a final concentration of 0.1 µg/µL for the PCR reaction mixture. Hot-start PCR is also recommended to increase the specificity. Try to use minimal amount of elute possible for downstream applications.Note: Please read through previous sections of this manual before using this

Mag-Bind® Stool DNA Kit - Centrifugal Protocol

Note: Please read through previous sections of this manual before using this protocol.

1. Prepare samples by following the standard protocol in previous sections.

2. For all binding, washing and elution steps. Instead to use the Magnetic Separation Device to magnetize the Mag-Bind® Particles, centrifuge the tube at 14,000 x g for 1 minute to collect the Mag-Bind® Particles.

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 HTR Reagent thoroughly. Add 100 µL to cleared supernatant. Mix by vortexing. Let sit for two minutes. Centrifuge at 14,000 x *g* for 1 minutes and transfer cleared supernatant to next step. Do not reuse Buffer SP2.
	Salt contamination	• Repeat the DNA isolation with a new sample. • Extend the incubation time with VHB Buffer. • Wash the Mag-Bind® Particles with ethanol.
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 Buffer SLX thoroughly. Use a commercial homogenizer if possible.
	Loss the Mag-Bind® Particles during operation	Carefully avoid remove the Mag-Bind® Particles during aspiration
	DNA remains bound to Mag-Bind® Particles	Increase elution volume and incubate on column at 65 °C for 5 minutes elution
	DNA washed off.	Make sure VHB Buffer and SPM Wash 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
	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 CND to 15 minutes before elution.