

Contents

Introduction.....	2
Overview.....	2
Storage and Stability.....	2
Kit Contents.....	3
Materials to Be Provided by User.....	3
Before Starting.....	3
Mag-Bind Stool DNA Protocol for Pathogen Detection.....	5
Mag-Bind Stool DNA Protocol for Human DNA Detection.....	8
Mag-Bind Stool DNA Centrifugation Protocol.....	11
Trouble Shooting Guide.....	12

Introduction

The E.Z.N.A.™ 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. E.Z.N.A.™ Mag-Bind Stool DNA Kit uses a unique HTR Reagent and SP2 Buffer that can remove inhibitory substances from stool samples.

If using the E.Z.N.A.™ 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 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.

Storage and Stability

Most components of the E.Z.N.A.™ Mag-Bind Stool DNA Kit should be stored at 22°C-25°C. OB Protease should be stored at 15-25°C. Mag-Bind® Particles Solution should be stored at 4 ° C for long-term use. During shipment or storage in cool ambient conditions, precipitates may form in Buffer DS. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Kit Contents

Product Number	M4015-00	M4015-01	M4015-02
Purification	5 Preps	50 Preps	200 Preps
MagSi Particle	120 µl	1200 µl	4.2 ml
Buffer SLX	10 mL	90 mL	2 x 160 mL
SP2 Buffer	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 mg	12 g	45 g
Elution Buffer *	2.0 mL	15 mL	60 mL
Instruction Booklet	1	1	1

*Elution Buffer is 10 mM Tris Hcl pH 8.5. Buffer MSL contains Chaotropic salts.

Before Starting

Please read the entire booklet to become familiar with the E.Z.N.A.™ Mag Bind Stool DNA Kit protocol.

- Dilute SPM Buffer with absolute ethanol as follows and store at room temperature.

M4015-00	Add 7 mL absolute (96%-100%) ethanol.
M4015-01	Add 70 mL (96%-100%) ethanol to each bottle.
M4015-02	Add 140 mL (96%-100%) ethanol to each bottle.

- Dilute Buffer VHB with absolute ethanol (96-100%).

M4015-00	Add 3 mL absolute (96%-100%) ethanol
M4015-01	Add 28 mL absolute (96%-100%) ethanol
M4015-02	Add 112 mL absolute (96%-100%) ethanol

Stool DNA Protocol (for human DNA detection)

Materials to be provided 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
 - Absolute (96%-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 thoroughly homogenized.**

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 SLX Mlus Buffer is added into the tube.
 2. **Add 400µl of SP2 Buffer and mix thoroughly by vortexing for 10 seconds.** Incubate on ice for 5 minutes.
 3. **Centrifuge at full speed ($\geq 14,000 \times g$) for 3 minutes** at room temperature.
 4. **Transfer 1 ml of cleared supernatant to a new 2 ml tube and add 200µl of HTR Reagent.** Mix the sample thoroughly by voretxing for 10 seconds.

Important: HTR reagent must be thoroughly suspended before being dispense from bottle. Tip: Use 1ml 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 ($>13,000 \times g$) 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 20µl of OB Protease(20mg/ml) and mix thoroughly by voretxing.
 8. **Add 300 µl of Bindin Buffer and 20 µl MagSi Particles. Mix by vortexing for 30 seconds.**
 9. Place the tube or plate on a magnetic separation device to magnetize the

magnetic particles. Completely aspirate the cleared supernatant by pipetting.

10. Remove the tube or plate containing the magnetic particles from the magnetic separation device. **Add 700µl of Buffer VHB/ethanol for each sample.**
11. Resuspend magnetic particles pellet 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 magnetic particles. Completely aspirate the cleared supernatant by pipetting
13. Remove the tube or plate containing the magnetic particles from the magnetic separation device. **Add 700µl of SPM Buffer for each sample.**
14. Resuspend magnetic particles pellet 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 magnetic particles by breaking up the magnetic particle pellet.
15. Completely aspirate the cleared supernatant by pipetting.
16. Remove the tube or plate containing the magnetic particles from the magnetic separation device. **Add 700µl of SPM Buffer for each sample.**
17. Resuspend magnetic particles pellet by vortexing or pepetting up and down.
18. Place the plate or tube onto a magnetic separation device to magnetize the magnetic particles.
19. Completely aspirate the cleared supernatant by pipetting.
20. Leave the tube to air dry on the magnetic separation device for 5-10.minutes. Remove any residue liquid from tube by pipetting.
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 ul Elution Buffer or water to elute DNA from magnetic particles.**
22. Resuspend magnetic particles by pipet up and down for 50 times or vortex for 3 minutes. Incubate 5-10 minutes at room temperature.

23. Place the tube or plate onto a magnetic separation device to magnetize the Mag-Si particles.
24. **Transfer the cleared supernatant containing purified DNA to a new 1.5 ml tube.**
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. The expected yield from a 20 mg sample is 8-35 µg genomic DNA, depending on type of tissue

Stool DNA Protocol (for pathogen detection)

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 SLX Mlus Buffer/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 200µl of Buffer SP2.** Mix the sample thoroughly 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 minute 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 1ml pipettor and cut off the end of 1ml tip to make it easier for pipetting the HTR reagent.
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 MagSi Particles. Mix by vortexing for 30 seconds.**
10. Plate the tube or plate on a magnetic separation device to magnetize the magnetic particles. Completely aspirate the cleared supernatant by pipetting.

11. Remove the tube or plate containing the magnetic particles from the magnetic separation device. **Add 700µl of Buffer VHB/ethanol Buffer for each sample.**
12. Resuspend magnetic particles pellet 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 magnetic particles by breaking up the magnetic particle pellet.
13. Remove the tube or plate containing the magnetic particles from the magnetic separation device. **Add 700µl of SPM Buffer for each sample.**
14. Resuspend magnetic particles pellet 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 magnetic particles by breaking up the magnetic particle pellet.
15. Completely aspirate the cleared supernatant by pipetting.
16. Remove the tube or plate containing the magnetic particles from the magnetic separation device. **Add 700µl of SPM Buffer for each sample.**
17. Resuspend magnetic particles pellet by vortexing or pipetting up and down.
18. Place the plate or tube onto a magnetic separation device to magnetize the magnetic particles
19. Completely aspirate the cleared supernatant by pipetting
20. Leave the tube to air dry on the magnetic separation device for 5-10 minutes. Remove any residue liquid from tube by pipetting.
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 50-100ul Elution Buffer or water to elute DNA from magnetic particles.**
22. Resuspend magnetic particles by pipet up and down for 50 times or vortex for 3 minutes. Incubate 5-10 minutes at room temperature.
23. Place the tube or plate onto a magnetic separation device to magnetize the

Mag-Si particles.

24. **Transfer the cleared supernatant containing purified DNA to a new 1.5 ml tube.**

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. The expected yield from a 20 mg sample is 8-35 µg genomic DNA, depending on type of tissue.

Tip: for maximum PCR robustness, it is recommended to add BSA to a final concentration of 01.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.

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 collect the Mag-Bind particles, centrifuge the tube at 14,000 x g for 1 minute collect the magnetic beads.

Troubleshooting Guide

Problem	Cause	Suggestions
Low DNA yield	Incomplete disruption of starting material.	Repeat the experiment with new sample, make sure the sample are completely interrupted and lysed.
	Sample stored incorrectly	Store the sample at -20°C
	Loss the Mag-Bind particle 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 min elution
	DNA washed off.	Dilute MGB Binding Buffer and SPM Wash Buffer by adding appropriate volume of absolute ethanol prior to use (page 3).
Problems in downstream applications	BSA no added to PCR mixture	Wash Buffer must be at room temperature.
	Ethanol carry-over	Dry the Mag-Bind® particle before elution.
	Add BSA to a final concentration of 0.1µg/mL to the PCR mixture.	Add BSA to a final concentration of 0.1µg/mL to the PCR mixture.
A260/280 ratio is low	inefficient elimination of inhibitory compounds	Repeat with a new sample, be sure to mix HTR Reagent thoroughly before use