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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 P2 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 P2 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.

Storage and Stability

Most components of the E.Z.N.A.™ Mag-Bind Stool DNA Kit should be stored at 22°C-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
Mag-Bind Particle B	60 µl	550 µl	2.2 ml
SLX Mlus Buffer	10 mL	100 mL	400 mL
Buffer DS	2 mL	10 mL	45 mL
P2 Buffer	3 mL	30 mL	125 mL
HTR Reagent	1.2 mL	12 mL	50 mL
Buffer MP	2 mL	12 mL	40mL
Buffer MSL *	4 mL	60 mL	250 mL
SPM Wash Buffer	3 mL	30 mL	60 mL
Proteinase K	3 mg	30 mg	4x 30 mg
Proteinase Storage Buffer	155 µl	3 mL	12 mL
Glass Beads	1.2 mg	12 g	45 g
Elution Buffer *	2.0 mL	15 mL	60 mL
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*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 **MP Buffer** with **absolute ethanol** (96-100%).

M4015-00	Add 3 mL absolute (96%-100%) ethanol
M4015-01	Add 18mL absolute (96%-100%) ethanol
M4015-02	Add 60mL absolute (96%-100%) ethanol

- Prepare Proteinase K stock solution as following:

M4015-00	Add 150 µl Proteinase Storage Buffer to vial
M4015-01	Add 1.5 mL Proteinase Storage Buffer to vial
M4015-02	Add 1.5 mL Proteinase Storage Buffer to vial

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 1.6 mL SLX Mlus Buffer. Vortex at maximum speed for 5 minute or until the stool sample is throughly 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 Buffer is added into the tube.

2. Add160 µl Buffer DS and vortex to mix. to mix.

2. Centrifuge at full speed (>14,000 x g) for 3 minutes to pellet the stool particles.

3. Transfer 1.5 ml of the supernatant into a new 2 mL tubes (not supplied).

Note: to make the pipetting easier for viscous stool sample, cut the end of pipet tips**.**

4. Add 550µl of P2 Buffer and mix throughly by vortexing for 10 seconds. Incubate on ice for 5 minutes.

5. Centrifuge at full speed (≥13,000 x g) for 3 minutes at room temperature.

6. Transfer 1.4 ml of cleared supernatant to a new 2 ml tube and add 200µl 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 1ml pipettor and cut off the end of 1ml tip to make it easier for pipetting the HTR reagent.

7. Incubate at room temperature for 2 minutes.

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

9. Transfer 600µl of supernatant into a new 2.0 ml centrifuge tube (not supplied). Add 20µl of Proteinase K (20mg/ml) and mix throughly by vorexting.

10. Add 600µl of MSL Buffer and mix by vortexing for 10 seconds.

11. Incubate at 70°C for 10 minutes. Mix sample twice during incubation by vortexing the tube. Centrifuge briefly to remove any liquid drop from the tube lid.

12. Add 780µl of absolute ethanol and 10 µl of Mag-Bind Particles B. Mix by pipetting 10-20 times. Incubate at room temperature for 5 minutes

13. Place the tube or plate on a magnetic separation device to magnetize the magnetic particles

14. Completely aspirate the cleared supernatant by pipetting.

15. Remove the tube or plate containing the magnetic particles from the magnetic separation device. Add 400µl of MP Buffer/ethanol for each sample.

16. Resuspend magnetic particles pellet by vortexing or pipetting. Incubate 3 minutes at room temperature. Mix by vortexing or pipetting few times during incubation.

17. Place the tube or plate onto a magnetic separation device to magnetize the magnetic particles

18. Completely aspirate the cleared supernatant by pipetting

19. Remove the tube or plate containing the magnetic particles from the magnetic separation device. Add 400µl of SPM Buffer

20. 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

21. Completely aspirate the cleared supernatant by pipetting.

22. Remove the tube or plate containing the magnetic particles from the magnetic separation device. Add 400µl of SPM Buffer

23. Resuspend magnetic particles pellet by vortexing or pepetting up and down

24. Place the plate or tube onto a magnetic separation device to magnetize the magnetic particles

25. Completely aspirate the cleared supernatant by pipetting

26. 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.

27. Remove the tube or plate from magnetic separation device. Add 50-200ul Elution Buffer or water to elute DNA from magnetic particles

28. Resuspend magnetic particles by pipet up and down for 50 times or vortex for 3 minutes. Incubate 5-10 minutes at room temperature

29. Place the tube or plate onto a magnetic separation device to magnetize the Mag-Bind™ particles.

30. 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 Buffer/Proteinase K is added into the tube.

2. Add 270 µL SLX Mlus Buffer Vortex at maxi speed for 5 minute or until the stool sample is throughly homogenized.

3. Add 30 µL Buffer DS followed by then adding 20 µL of Proteinase K Vortex to mix

4. 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.

5. Incubate the sample on ice for 2 minutes.

6. Add 100µl of Buffer P2. Mix the sample throughly by voretxing the tube for 30 seconds.

7. Incubate the sample on ice for 5 minutes.

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

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

10. Add 200 µL of HTR Reagent. Mix the sample by vortexing the tube for 10 seconds.

    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.

11. Incubate at room temperature for 2 minutes.

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

13. Transfer 250 µL supernatant to a new 2.0 ml tube.

14. Optional: If RNA-free DNA is required, add 10 µl RNase A and mix throughly by vortexing. Incubate at 70°C for 3 minutes.

15. Add 250 µl of MSL Buffer followed by 325 µl of absolute ethanol to the lysate. Add 10 µl of Mag Bind Particles B. Incubate at room temperature for 5 minutes Place the tube or plate on a magnetic separation device to magnetize the magnetic particles.

15. Completely aspirate the cleared supernatant by pipetting.

16. Remove the tube or plate containing the magnetic particles from the magnetic separation device. Add 400µl of MP/ethanol Buffer for each sample.

17. 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.

18. Place the tube or plate onto a magnetic separation device to magnetize the magnetic particles.

19. Completely aspirate the cleared supernatant by pipetting.

20. Remove the tube or plate containing the magnetic particles from the magnetic separation device. Add 400µl of SPM Buffer.

21. 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.

22. Completely aspirate the cleared supernatant by pipetting.

23. Remove the tube or plate containing the magnetic particles from the magnetic separation device. Add 400µl of SPM Buffer.

24. Resuspend magnetic particles pellet by vortexing or pepetting up and down.

25. Place the plate or tube onto a magnetic separation device to magnetize the magnetic particles.

26. Completely aspirate the cleared supernatant by pipetting.

27. 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.

28. Remove the tube or plate from magnetic separation device. Add 50-200ul Elution Buffer or water to elute DNA from magnetic particles.

29. Resuspend magnetic particles by pipet up and down for 50 times or vortex for 3 minutes. Incubate 5-10 minutes at room temperature.

30. Place the tube or plate onto a magnetic separation device to magnetize the Mag-Bind™ particles.

31. Transfer the cleared supernatant containing purified DNA to a new 1.5 ml tube or 96-well plate

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 65ºC 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 throughly before use