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Introduction

E.Z.N.A.[®] Stool DNA Miniprep Kits allow 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 90 minutes. The system combines the reversible nucleic acid-binding properties of HiBind[®] matrix with the speed and versatility of spin column technology 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.[®] Stool DNA Miniprep Kit's STL2 Buffer is specially designed to remove inhibitory substances from stool samples.

If using the E.Z.N.A.[®] Stool DNA Miniprep 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 after a heat-freeze step. Contaminants are further removed by isopropanol precipitation of DNA. Binding conditions are then adjusted and the sample is applied to a HiBind[®] DNA spin-column. 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

All components of the E.Z.N.A.[®] Stool DNA Miniprep Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by incubating the buffers at 65°C and gently shaking their containers.

Kit Contents

Product Number	D4015-00	D4015-01	D4015-02
HiBind® DNA Columns	5	50	200
2 ml Collection Tubes	15	150	600
STL1 Buffer	0.7 ml	7 ml	22 ml
STL2 Buffer	2.5 ml	10 ml	40 ml
STL3 Buffer	1.0 ml	10 ml	40 ml
HB Buffer	3 ml	30 ml	110 ml
Proteinase K	3 mg	30mg	4 x 30 mg
DNA Elution Buffer	1.5 ml	15 ml	50 ml
Wash Buffer Concentrate	12 ml	48 ml	2 x 60 ml
Instruction Booklet	1	1	1

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.® Stool DNA Miniprep Kit procedures.
- Prepare an STL1 Buffer stock solution by adding 10µl 2-mercaptoethanol per 1 ml STL1 Buffer before use. Each sample will require 70 µl of this solution.
- Dilute Wash Buffer Concentrate with ethanol as follows and store at room temperature:

D4015-00 Add 18 ml absolute (96%-100%) ethanol.

D4015-02 Add 72 ml absolute (96%-100%) ethanol to each bottle.

D4015-02 Add 90 ml absolute (96%-100%) ethanol to each bottle.

- Prepare Proteinase K stock solution as follows:

D4015-00 Add 125µl Elution Buffer to the vial

D4015-01 Add 1.25 ml Elution Buffer to the vial

D4015-02 Add 1.25 ml Elution Buffer to the vial

Stool DNA Miniprep Protocol (for pathogen detection)

Materials to be provided by user:

- Microcentrifuge capable of at least 14,000 x g
- Nuclease-free 1.5 ml or 2 ml microfuge tubes
- Water bath equilibrated to 65°C
- Equilibrate sterile dH₂O water or 10 mM Tris pH 9.0 at 65° C
- 2-mercaptoethanol
- PBS Buffer
- Isopropyl alcohol (isopropanol)
- Absolute (96%-100%) ethanol
- RNase A stock solution at 20 mg/ml

1. Weigh up to 200 mg stool sample in a 2 ml centrifuge tube and place the tube on ice. Add 630 µl PBS Buffer followed by 70 µl STL1 /2-mercaptoethanol solution. Vortex continuously 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. If the sample is frozen, use a spatula to scrape the sample into the tube. Do not thaw the frozen sample until the STL1 Buffer/2-mercaptoethanol is added into the tube.

2. Transfer 800 µl of the stool lysate into a new 2 ml tubes (not supplied). **Note:** to make the pipetting easier for viscous stool sample, cut the end of pipet tips.
3. Incubate at 70°C for 5 min (7 min if frozen). Mix sample twice during incubation by vortexing the tube. For gram positive bacterial, this lysis temperature can be increased to 95°C.
4. Incubate the sample on ice for 5 minutes.
5. Vortex the sample for 30 seconds, then centrifuge at full speed in a microcentrifuge for 5 minute to pellet the stool particles.
6. Carefully aspirate 400µl supernatant to a new 2 ml microfuge tube (not supplied), making sure not to disturb the pellet or transfer any debris.
7. Add 400µl of STL2 Buffer followed by 25µl of Proteinase K. Mix the sample by vortexing.
8. Incubate at 70°C for 10 minutes. Centrifuge briefly to collect any drops from the tube lid.
9. To the lysate, add equal volume (800µl) of chloroform:isoamyl alcohol (24:1) and vortex to mix. Centrifuge at 10,000 x g for 2 min at room temperature. Carefully transfer the **upper** aqueous phase to a clean 1.5 ml microfuge tube. Avoid the milky interphase containing contaminants and inhibitors.
10. Add 0.7 x vol isopropanol and mix to precipitate DNA. Incubate at room

temperature 2 min and centrifuge at 10,000 x g for 10 min.

11. Carefully discard as much supernatant as possible without disturbing the pellet. Air dry 2 min at room temperature.
12. Resuspend DNA pellet in 300 µl sterile deionized water. This may require incubation at 60°C-70°C for 10 minutes or more. Add 10 µl RNase A (20 mg/ml) and mix.
13. Adjust binding conditions by adding 150 µl STL3 Buffer followed by 300 µl absolute ethanol and vortex to mix. Apply entire mixture, including any precipitation that may have formed, to a HiBind® DNA column inserted in a 2 ml collection tube (supplied). Centrifuge at ≥ 10,000 x g for 1 min at room temperature. Discard flow-through liquid and collection tube.
14. Place column into a new 2 ml collection tube (supplied) and wash by adding 500 µl HB Buffer. Centrifuge at 10,000 x g for 30 seconds. Discard the flow-through and collection tube.
15. Place column into new 2 ml collection tube (supplied) and wash by adding 700 µl DNA Wash Buffer diluted with absolute ethanol. Centrifuge 10,000 x g 1 min as above. Discard flow-through liquid and reuse collecting tube in next step.
16. Repeat step 14 with a second 700 µl DNA Wash Buffer. Discard liquid and re-insert the column to the empty collection tube. Centrifuge the column at full speed for 1 min at room temperature. This step is critical for removal of traces of ethanol that will otherwise interfere with downstream applications (such as agarose gel electrophoresis of high molecular weight DNA).
17. Place column into a clean 1.5 ml microfuge tube (not supplied). To elute DNA add 100-200µl of DNA Elution Buffer (or 10 mM Tris buffer, pH 9.0) preheated to 65°C-70°C directly onto the HiBind® matrix. Incubate 2 min at room temperature. Centrifuge at full speed for 1 min to collect DNA.

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.

Stool DNA Miniprep 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 or 2 ml microfuge tubes
- Water bath equilibrated to 65°C
- Equilibrate sterile dH₂O water or 10 mM Tris pH 9.0 at 65°C
- 2-mercaptoethanol
- PBS Buffer
- Isopropyl alcohol (isopropanol)

- Absolute (96%-100%) ethanol
- RNase A stock solution at 20 mg/ml

1. Weigh up to 200 mg stool sample in a 2 ml centrifuge tube and place the tube on ice. Add 900 µl PBS Buffer followed by 100 µl STL1 /2-mercaptoethanol solution. Vortex continuously 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. If the sample is frozen, use a spatula to scrape the sample into the tube. Do not thaw the frozen sample until the STL1 Buffer/2-mercaptoethanol is added into the tube.

2. Centrifuge at full speed for 3 minutes to pellet the stool particles.
3. Transfer 600 µl of the stool lysate 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. Incubate at 70°C for 5 min (7 min if frozen). Mix sample twice during incubation by vortexing the tube.
5. Incubate the sample on ice for 5 minutes.
6. Vortex the sample for 30 seconds, then centrifuge at full speed in a microcentrifuge for 2 minutes.
7. Carefully aspirate 450µl supernatant to a new 2 ml microfuge tube, making sure not to disturb the pellet or transfer any debris.
8. Add 450µl of STL2 Buffer followed by 25µl of Proteinase K. Mix the sample by vortexing.
9. Incubate at 70°C for 10 minutes. Centrifuge briefly to collect any drops from the tube lid.
10. To the lysate, add equal volume (900µl) of chloroform:isoamyl alcohol (24:1) and vortex to mix. Centrifuge at 10,000 x g for 2 min at room temperature. Carefully transfer the **upper** aqueous phase to a clean 1.5 ml microfuge tube. Avoid the milky interphase containing contaminants and inhibitors.
11. Add 0.7 x vol isopropanol and mix to precipitate DNA. Incubate at room temperature 2 min and centrifuge at 10,000 x g for 10 min.

12. Carefully discard as much supernatant as possible without disturbing the pellet. Air dry 2 min at room temperature.
13. Resuspend DNA pellet in 300 µl sterile deionized water. This may require incubation at 60°C-70°C for 10 minutes or more. Add 10 µl RNase A (20 mg/ml) and mix.

14. Adjust binding conditions by adding 150 µl STL3 Buffer followed by 300 µl absolute ethanol and vortex to mix. Apply entire mixture, including any precipitation that may have formed, to a HiBind® DNA column inserted in a 2 ml collection tube (supplied). Centrifuge at $\geq 8,000 \times g$ for 1 min at room temperature. Discard flow-through liquid and collection tube.
15. Place column into a new 2 ml collection tube (supplied) and wash by adding 500 µl HB Buffer. Centrifuge at 10,000 $\times g$ for 30 seconds. Discard the flow-through and collection tube.
16. Place column into new 2 ml collection tube (supplied) and wash by adding 700 µl DNA Wash Buffer diluted with absolute ethanol. Centrifuge 10,000 $\times g$ for 1 min as above. Discard flow-through liquid and reuse collecting tube in next step.
17. Repeat step 14 with a second 700 µl DNA Wash Buffer. Discard liquid and re-insert the column to the empty collection tube. Centrifuge the column at full speed for 1 min at room temperature. This step is critical for removal of traces of ethanol that will otherwise interfere with downstream applications (such as agarose gel electrophoresis of high molecular weight DNA).
18. Place column into a clean 1.5 ml microfuge tube (not supplied). To elute DNA add 50 µl - 100 µl of DNA Elution Buffer (or 10 mM Tris buffer, pH 9.0) preheated to 60°C-70°C directly onto the HiBind® matrix. Incubate 2 min at room temperature. Centrifuge at full speed for 1 min to collect DNA.

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.

Troubleshooting

Problem	Cause	Suggestions
A260/280 ratio is low	inefficient elimination of inhibitory compounds	Repeat the DNA isolation with a new sample, be sure to mix the sample with STL2 Buffer thoroughly.
	DNA pellet not completely dissolved before applying sample to column.	Ensure that DNA is dissolved in water before adding Buffer STL3 and ethanol. This may need repeated incubation at 65°C and vortexing.
	No ethanol added to the lysate before loading to the column	Repeat the DNA isolation with a new sample.
	DNA wash Buffer prepared with lower percentage ethanol	prepare DNA Wash Buffer with 96-100% ethanol

A260/280 ratio is high	RNA contamination	Be sure to treat the sample with RNase A in step 9.
Low DNA yield or no DNA eluted	Sample stored incorrectly	Sample should be stored at 4°C or -20°C
	Poor homogenization of sample.	Repeat the DNA isolation with a new sample, be sure to mix the sample with PBS/STL1 thoroughly.
	No ethanol added to the lysate before loading to the column	Repeat the DNA isolation with a new sample
	DNA washed off.	Dilute Wash Buffer Concentrate by adding appropriate volume of absolute ethanol prior to use (page 3).
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 reaction	Dilute the DNA elute used in the downstream application if possible.
	No-specific bands in downstream PCR	Use hot-start Taq polymerase mixture
	inhibitory substance in the eluted DNA.	Check the A260/280 ratio Dilute the elute to 1:50 if necessary
	Ethanol residue in the elute	Be sure to completely dry the column before elution
Little or no supernatant after initial centrifuge step	Insufficient centrifugal force	Check the centrifugal force and increase the centrifugal time if necessary
sample can not pass through the column	Clogging column	Check the centrifugal force and increase the time of centrifugation

For assistance or to place orders, please contact Omega Bio-Tek:

Tel: 800-832-8896 (toll-free) or 770-931-8400 (local/international)

Fax: 888-624-1688 (toll-free) or 770-931-0230 (local/international)

E-mail: info@omegabiotek.com

Web address: www.omegabiotek.com