Problem	Cause	Suggestions
A260/230 ratio is low	inefficient elimination of inhibitory compounds	Repeat with a new sample, be sure to mix the sample with HTR Reagent throughly.
	No ethanol added to the lysate before loading to the column	Repeat the DNA isolation with a new sample.
	No ethanol added to DNA Wash Buffer	prepare DNA Wash Buffer with 96-100% ethanol
A260/280 ratio is high	RNA contamination	Be sure to treat the sample with RNase A according to the protocol.
Low DNA yield or no DNA eluted	Sample stored incorrectly	Sample should be store at 4°C or -20°C
	Poor homogenization of sample.	Repeat with a new sample, be sure to mix the sample with Buffer SP1 throughly.
	DNA washed off.	Dilute DNA Wash Buffer Concentrate by adding absolute ethanol prior to use (page 4).
Problems in downstream applications	BSA no added to PCR mixture	Add BSA to a final concentration of 0.1μg/mL to the PCR mixture.
	Too much DNA inhibit PCR reaction	Diluted 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 A260/230 ration Diluted 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

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the liquid drop.

9. **Add 250µl of Elution Buffer and resuspend the pellet by vortexing for 20 seconds. Incubate the tube at 70°C. Vortex the tube twice during incubation.**

10. **Optional: If RNA-free DNA is required, add 10 µl RNase A and mix throughly by vortexing.**

11. **Transfer the sample into a new 1.5 ml microtube 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.

12. **Incubate at room temperature for 2 minutes.**

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

14. Transfer 250µl of supernatant into a new 2.0 ml centrifuge tube (not supplied). Add 10µl of Proteinase K (20mg/ml) and mix throughly by vorexting.

15. Add 250µl of BL Buffer and mix by vortexing for 10 seconds.

16. **Incubate at 70ºC for 5 minutes.** Mix sample twice during incubation by vortexing the tube. Centrifuge briefly to remove any liquid drop from the tube lid.

17. Add 250µl of absolute ethanol and mix throughly by vortexing for 10 seconds. Centrifuge briefly to remove any liquid drop from the tube lid (optional).

18. **Apply entire sample including any precipitation that may have formed, to a HiBind® DNA column assembled in a 2 mL collecting tube (supplied).** Centrifuge at full speed (>13,000 x g) for 1 min at room temperature. Discard flow-through liquid and collection tube.

19. **Place column into a new 2 mL collection tube (supplied) and wash the column by adding 500 µL HB Buffer.** Centrifuge at full speed (>13,000 x g) for 1 min. Discard flow-through liquid and collecting tube in next step.

Kit Contents

Product Number	D4015-00	D4015-01	D4015-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind® DNA Columns	5	50	200
2 mL Collection Tubes	15	150	600
Buffer SP1	12 ml	100 ml	2 x 200 ml
Buffer SP2	3 ml	30 ml	120 ml
HTR Reagent	1.2 ml	12 ml	50 ml
Buffer BL	5 ml	35 ml	125 ml
Buffer HB	5 ml	30 ml	120 ml
Glass Beads	1.2 g	12 g	45 g
Proteinase K	3 mg	30 mg	120 mg
Protease Storage Buffer	200 ul	1.8 ml	7 ml
Elution Buffer	5 ml	30 ml	100 ml
DNA Wash Buffer	2 ml	20 ml	3 x 20 ml
Instruction Booklet	1	1	1

Materials to be provided by user

- Microcentrifuge capable of at least 13,000 x g
- Nuclease-free 1.5 mL or 2 mL microfuge tubes
- Water bath or heating block preset to 65ºC and 70°C
- Equilibrate sterile dH_2O or 10 mM Tris pH 8.5 at 65°C.
- Absolute (96%-100%) ethanol
- RNase A stock solution at 20 mg/mL
- Isopropanol (100%)

13. **Apply 600µl of sample from step 12 including any precipitation that may have formed, to a HiBind® DNA column assembled in a 2 mL collecting tube (supplied).** Centrifuge at full speed (>13,000 x g) for 1 min at room temperature. Discard flow-through liquid and collection tube.

14. Place the HiBind® **into a new 2 ml collection tube. Apply another 600µl of sample from step 12 to the HiBind® DNA column.** Centrifuge at full speed (>13,000 x g) for 1 min at room temperature. Discard flow-through liquid and re-use collection tube.

15. Repeat the step 14 by apply the third aliquot of 600µl lysate into the **HiBind® DNA column.**

16. **Place column into a new 2 mL collection tube (supplied) and wash the column by adding 500 µL HB Buffer.** Centrifuge at full speed (>13,000 x g) for 1 min. Discard flow-through liquid and collecting tube in next step.

17. Place the HiBind® DNA column into a new collection tube (supplied). Add 750µl of DNA Wash Buffer into the column and centrifuge at full speed (>13,000 x g) for 1 min. Discard liquid and re-insert the column to the empty collection tube.
Note: DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle and page 3.

18. **Centrifuge the column at full speed (>13,000 x g) for 2 min at room temperature to dry the column.** ***This step is critical in removing traces of ethanol that will interfere with downstream applications***.

19. **Place column into a clean 1.5 mL microfuge tube (not suplied).** To elute DNA **add 200 µL of Elution Buffer** (10 mM Tris buffer, pH 8.5) preheated to 60°C-70°C directly onto the HiBind® matrix. **Allow to soak for 2 min at room temperature. Centrifuge at full spped (≥13,000 x g) for 1 min to Elute DNA.**

Tip: for maximum PCR robustness, it is recommended to add BSA to a final concentration of 0.1µg/ 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.

Protocol for Isolating DNA from Large Volume of Stool

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 SP1 Buffer/Proteinase K is added into the tube.

2. **Add 300 µL Buffer SP1 followed by then adding 10 µL of Proteinase K solution.** Vortex at maxi speed for 5 minute or until the stool sample is throughly homogenized.

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 90°C for 5 minutes.

4. **Incubate the sample on ice for 2 minutes.**

5. **Add 100 µl of Buffer SP2. Mix the sample throughly by voretxing the tube for 30 seconds.**

6. **Incubate the sample on ice for 5 minutes.**

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

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

9. **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.

10. **Incubate at room temperature for 2 minutes**.

11. **Centrifuge at full speed (>13,000 x g) for 2 minutes to pellet the inhibitors absorb to HTR Reagent.**
12. **Transfer 250 µL supernatant to a new 2.0 ml tube.**

Introduction

The E.Z.N.A.® 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 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 allowing 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 Kit uses an unique HTR Reagent and SP2 Buffer that can remove inhibitory substances from stool samples..

If using the E.Z.N.A.® 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 by adding BL Buffer and the sample is applied to a HiBind® DNA spin-column. Two rapid wash steps remove trace contaminants, and pure DNA is eluted with 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 Kit, except for Proteinase K should be stored at 22°C-25°C. Once reconstituted in water, Proteinase K should be stored at -20°C. Under these conditions, DNA has successfully been purified and used for PCR after 24 months of storage. During shipment, or storage in cool ambient conditions, precipitates may form in some buffers. It is possible to dissolve such deposits by incubation the solution at 65°C.

20. Place the HiBind® DNA column into a new collection tube (supplied). Add 750µl of DNA Wash Buffer into the column and centrifuge at full speed (>13,000 x g) for 1 min. Discard liquid and re-insert the column to the empty collection tube.
    Note: DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle and page 3. If refrigerated, the diluted DNA Wash Buffer must be brought to room temperature before use.

21. **Centrifuge the column at full speed (>13,000 x g) for 2 min at room temperature to dry the column.**
    This step is critical in removing traces of ethanol that will interfere with downstream applications.

22. **Place column into a clean 1.5 mL microfuge tube (not suplied).** To elute DNA **add 200 µL of Elution Buffer** (10 mM Tris buffer, pH 8.5) preheated to 60°C-70°C directly onto the HiBind® matrix. **Allow to soak for 2 min at room temperature. Centrifuge at full spped (≥13,000 x g) for 1 min to Elute 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.

Troubleshooting Guide

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.® Stool DNA Kit protocol..

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

D4015-00	Add 8 mL absolute (96%-100%) ethanol.
D4015-01	Add 80 mL (96%-100%) ethanol to each bottle.
D4015-02	Add 80 mL (96%-100%) ethanol to each bottle.

- Prepare Proteinase K stock solution as following

D4015-00	Add 150 ul Protease Storage Buffer to the vial
D4015-01	Add 1.5 ml Protease Storage Buffer to the vial
D4015-02	Add 6.0 ml Protease Storage Buffer to each vial
Vortex vial briefly prior to use. We recommend that you aliquot and store vials of reconstituted protease at -20°C.	

The following protocol is designed when the targeting DNA is not distributed homogenous in the stool sample. Use large volume of starting material will enhance the chances of isolating DNA from lower titer sources in the stool sample. Please note that excess volume of reagents will be required to use this protocol. Additional reagents can be purchased separately, call Omega Bio-tek or your local distributor for order information.

Materials to be provided by user

- Microcentrifuge capable of at least 13,000 x g
- Centrifuge capable of at least 3,000 x g
- Centrifuge with adaptor for 15 or 50 ml tube capable of at least 4,000 x g
- Nuclease-free 15ml or 50 ml centrifuge tubes
- Nuclease-free 1.5 mL or 2 mL microfuge tubes
- Water bath or heating block preset to 65ºC and 70˚C
- Equilibrate sterile dH_2O or 10 mM Tris pH 8.5 at 65ºC.
- Absolute (96%-100%) ethanol
- RNase A stock solution at 20 mg/mL
- Isopropanol (100%)

1. **Add stool sample into a 15 mL or 50 ml centrifuge tube (not supplied) and place the tube on ice. Add 10 volume of Buffer SP1 (i.e. add 10 ml Buffer SP1 to 1 gram of stool sample). Vortex at maximum speed for 5 minute or until the stool sample is throughly homogenized**.

2. **Centrifuge at full speed (>4,000 x g) for 15 minutes to pellet the stool particles.**

3. **Transfer the supernatant into a new 15ml or 50 mL tubes** (not supplied).
   Note: *to make the pipetting easier for viscous stool sample, cut the end of pipet tips.*

4. **Add 1/3 volume of SP2 Buffer and mix throughly by vortexing for 20 seconds. Incubate on ice for 5 minutes.**

5. **Centrifuge at full speed (≥3,000 x g) for 10 minutes at room temperature.**

6. **Transfer cleared supernatant to a new 15 or 50 ml tube and add equal volume of isopropanol. Mix throughly by invert the tube 10 times.**

7. **Centrifuge at full speed (≥3,000 x g) for 10 minutes at room temperature.**
8. **Discard the supernatant and invert the tube on a absorbent paper to drain**

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

14. **Add 250 µl of BL Buffer followed by 250µl of absolute ethanol to the lysate. Miix sample throughly by vortexing the tube for 10 seconds.**

15. **Apply entire sample from step 14 including any precipitation that may have formed, to a HiBind® DNA column assembled in a 2 mL collecting tube (supplied).** Centrifuge at full speed (>13,000 x g) for 1 min at room temperature. Discard flow-through liquid and collection tube.

16. **Place column into a new 2 mL collection tube (supplied) and wash the column by adding 500 µL HB Buffer.** Centrifuge at full speed (>13,000 x g) for 1 min. Discard flow-through liquid and collecting tube in next step.

17. Place the HiBind® DNA column into a new collection tube (supplied). Add 750µl of DNA Wash Buffer into the column and centrifuge at full speed (>13,000 x g) for 1 min. Discard liquid and re-insert the column to the empty collection tube.

    Note: DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle and page 3. If refrigerated, the diluted DNA Wash Buffer must be brought to room temperature before use.

18. **Centrifuge the column at full speed (>13,000 x g) for 2 min at room temperature to dry the column.**

    This step is critical in removing traces of ethanol that will interfere with downstream applications.

19. **Place column into a clean 1.5 mL microfuge tube (not suplied).** To elute DNA **add 200 µL of Elution Buffer** (10 mM Tris buffer, pH 8.5) preheated to 60ºC-70ºC directly onto the HiBind® matrix. **Allow to soak for 2 min at room temperature. Centrifuge at full spped (≥13,000 x g) for 1 min to Elute 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 Protocol (for human DNA detection)

1. **Weigh up to 200 mg stool sample in a 2 mL centrifuge tube (not supplied) and place the tube on ice. Add 1.8 mL Buffer SP1. 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 Buffer SP1 is added into the tube.

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 500µl of SP2 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 BL Buffer and mix by vortexing for 10 seconds.**

11. **Incubate at 70ºC 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 600µl of absolute ethanol and mix throughly by vortexing for 10 seconds. Centrifuge briefly to remove any liquid drop from the tube lid (optional).