E.Z.N.A.® Stool DNA Kit

D4015-00	5 preps
D4015-01	50 preps
D4015-02	200 preps

April 2013

E.Z.N.A.® Stool DNA Kit

Table of Contents

Manual Revision: April 2013

OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction and Overview

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 stool samples can be processed in less than 60 minutes. The system combines the reversible nucleic acid-binding properties of our 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.

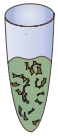
Stool samples typically contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions. E.Z.N.A.® Stool DNA Kit uses an unique HTR Reagent and P2 Buffer that can remove inhibitory substances from stool samples.

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 P2 Buffer after a heat-freeze step. Contaminants are further removed by HTR Reagent during a quick centrifuge step. Binding conditions are adjusted by adding BL Buffer and the sample is applied to a HiBind® DNA Mini Column. Two rapid wash steps remove trace contaminants, and pure DNA is eluted with Elution Buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

New in this Edition:

- Equilibration Buffer (used in the Troubleshooting section) is no longer included with this kit.
- Equilibration Buffer can be replaced with 3M NaOH provided by the user.
- Proteinase K is now supplied in a liquid form eliminating the resuspension step prior to use. Proteinase K Solution can be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.

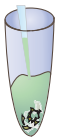
Illustrated Protocol



Lyse Samples

Precipitate Polysaccharides

Remove Inhibitors



Transfer Supernatant and Bind DNA

Wash 2X

Dry

Elute

Kit Contents

Product Number	D4015-00	D4015-01	D4015-02
Preparations	5 Preps	50 Preps	200 Preps
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	15	150	600
SLB Buffer	12 mL	100 mL	400 mL
DS Buffer	1.2 mL	10 mL	40 mL
SP2 Buffer	3 mL	30 mL	120 mL
HTR Reagent	1.2 mL	12 mL	50 mL
BL Buffer	5 mL	35 mL	125 mL
Glass Beads	1.2 g	12 g	45 g
VHB Buffer	2.2 mL	15 mL	66 mL
Proteinase K Solution	150 µL	1.5 mL	6.0 mL
Elution Buffer	5 mL	30 mL	100 mL
DNA Wash Buffer	2 mL	20 mL	3 x 20 mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® Stool DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. HTR Reagent should be stored at 2-8°C for long-term use. Proteinase K can be stored at room temperature for up to 12 months. For long-term storage, store Proteinase K at 2-8°C. All other components can be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 65°C and gently shaking.

- Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D4015-00	8 mL
D4015-01	80 mL
D4015-02	80 mL per bottle

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

Kit	100% Ethanol to be Added
D4015-00	2.8 mL
D4015-01	19.1 mL
D4015-02	84 mL

E.Z.N.A.® Stool DNA Kit Protocol - Pathogen Detection

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 2 mL microcentrifuge tubes
- Nuclease-free1.5 mL microcentrifuge tubes
- Water baths, heat blocks, or incubators capable of 65°C and 70°C
- Vortexer
- 100% ethanol
- Ice bath
- Optional: RNase A stock solution at 20 mg/mL
- Optional: Incubator capable of 95°C

Before Starting:

- Prepare ice bath
- Heat Elution Buffer to 65°C
- Set a water bath, heat block, or incubator to 70°C
- Prepare DNA Wash Buffer and VHB Buffer according to the Preparing Reagent section on Page 5
- Optional: for gram-positive bacteria set an incubator to 95°C

1. Add up to 200 mg stool sample in a 2 mL microcentrifuge tube containing 200 mg glass beads. Place the tube on ice.

   Note: If the sample is liquid, add 200 µL 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 SLB Buffer is added into the tube.

2. Add 540 µL SLB Buffer. Vortex at maximum speed for 10 minutes or until the stool sample is thoroughly homogenized.

   Note: We recommend a mechanical disruptor instrument such as the SPEX Geno/Grinder 2010 or a flat bed vortexer with tape.

3. Add 60 µL DS Buffer and 20 µL Proteinase K Solution. Vortex or pipet up and down to mix thoroughly.

4. Incubate at 70°C for 10 minutes (13 minutes if frozen). Vortex the sample twice during incubation.

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

5. Add 200 µL SP2 Buffer. Vortex at maximum speed for 30 seconds.

6. Let sit on ice for 5 minutes.

7. Centrifuge at full speed (≥13,000 x *g*) for 5 minutes.

8. Carefully aspirate 400 µL supernatant to a new 1.5 mL microcentrifuge tube (not supplied). Do not to disturb the pellet or transfer any debris.

9. Add 200 µL HTR Reagent. Vortex at maximum speed for 10 seconds.

   Note: HTR Reagent must be thoroughly resuspended before use. Cut the end of a 1 mL tip to make it easier to pipet the HTR Reagent.

10. Let sit at room temperature for 2 minutes.

11. Centrifuge at maximum speed for 2 minutes.

12. Transfer 250 µL supernatant to a new 1.5 mL microcentrifuge tube.

Optional: If RNA-free DNA is required, add 10 µL RNase A (not included). Vortex to mix thoroughly. Incubate at 37°C for 3 minutes. Continue to Step 13.

13. Add 250 µL BL Buffer and 250 µL 100% ethanol. Vortex at maximum speed for 10 seconds.

14. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

15. Transfer the entire sample from Step 13, including any precipitates that may have formed, to the HiBind® DNA Mini Column.

16. Centrifuge at maximum speed for 1 minute.

17. Discard the filtrate and the collection tube.

18. Transfer the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

19. Add 500 µL VHB Buffer.

    Note: VHB Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 5 for instructions.

20. Centrifuge at maximum speed for 30 seconds.

21. Discard the filtrate and reuse the collection tube.

22. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 5 for instructions.

23. Centrifuge at maximum speed for 1 minute.

24. Discard the filtrate and reuse collection tube.

25. Repeat Steps 22-24 for a second DNA Wash Buffer wash step.

26. Centrifuge at maximum speed for 2 minutes to dry the column.

    Note: It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

27. Transfer the column into a clean 1.5 mL microcentrifuge tube.

28. Add 100-200 µL Elution Buffer heated to 65°C directly to the center of the HiBind® matrix.

29. Let sit at room temperature for 2 minutes.

30. Centrifuge at maximum speed for 1 minute.

31. Store DNA at -20°C.

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

E.Z.N.A.® Stool DNA Kit Protocol - Human DNA Detection

Materials and Equipment to be Supplied by User:

- Centrifuge with adaptor for 15 mL centrifuge tubes capable of 4,000 x *g*
- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 mL and 2 mL microcentrifuge tubes
- 15 mL centrifuge tubes
- Water baths, heat blocks, or incubators capable of 65°C and 70°C
- Vortexer
- 100% ethanol
- Ice bath
- Optional: RNase A stock solution at 20 mg/mL
- Optional: Incubator capable of 95°C

Before Starting:

- Prepare ice bath
- Heat Elution Buffer to 65°C
- Set a water bath, heat block, or incubator to 70°C
- Prepare DNA Wash Buffer and VHB Buffer according to the Preparing Reagent section on Page 5
- Optional: for gram-positive bacteria set an incubator to 95°C

1. Add up to 200 mg stool sample in a 15 mL centrifuge tube (not supplied) and place the tube on ice. Add 1.6 mL SLB Buffer. Vortex at maximum speed for 1 minute or until the stool sample is completely homogenized.

   Note: If the sample is liquid, add 200 µL 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 SLB Buffer is added into the tube.

2. Add 180 µL DS Buffer. Invert 5 times to mix.

3. Centrifuge at maximum speed (≥4,000 x *g*) for 3 minutes.

4. Transfer 1.5 mL supernatant into a clean 15 mL centrifuge tube.

5. Add 600 µL SP2 Buffer. Vortex at maximum speed for 10 seconds.

6. Let sit on ice for 5 minutes.

7. Centrifuge at maximum speed (≥4,000 x *g*) for 3 minutes.

8. Transfer 600 µL cleared supernatant to a new 2 mL microcentrifuge tube.

9. Add 200 µL HTR Reagent. Vortex at maximum speed for 10 seconds.

   Note: HTR Reagent must be thoroughly resuspended before use. Cut the end of a 1 mL tip to make it easier to pipet the HTR Reagent.

10. Let sit at room temperature for 2 minutes.

11. Centrifuge at maximum speed for 2 minutes.

12. Transfer 600 µL supernatant into a new 2.0 mL microcentrifuge tube.

13. Add 20 µL Proteinase K Solution. Vortex to mix thoroughly.

14. Add 600 µL BL Buffer. Vortex at maximum speed for 10 seconds.

15. Incubate at 70°C for 10 minutes. Vortex the sample twice during incubation.

16. Centrifuge briefly to remove any liquid drops from the tube lid.

17. Add 600 µL 100% ethanol. Vortex at maximum speed for 10 seconds.

18. Centrifuge briefly to remove any liquid drops from the tube lid.

19. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

20. Transfer 600 µL sample from Step 18, including any precipitation that may have formed, to the HiBind® DNA Mini Column.

21. Centrifuge at maximum speed for 1 minute.

22. Discard the filtrate and reuse collection tube.

23. Repeat Steps 20-22 until all of the sample has been transferred to the HiBind® DNA Mini Column.

24. Transfer the HiBind® DNA Mini Column to a new 2 mL Collection Tube.

25. Add 500 µL VHB Buffer.

    Note: VHB Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 5 for instructions.

26. Centrifuge at maximum speed for 30 seconds.

27. Discard the filtrate and reuse collection tube.

28. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 5 for instructions.

29. Centrifuge at maximum speed for 30 seconds.

30. Discard the filtrate and reuse collection tube.

31. Repeat Steps 28-30 for a second DNA Wash Buffer wash step.

32. Centrifuge at maximum speed for 2 minutes to dry the column.

    Note: It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

33. Transfer the column into a clean 1.5 mL microcentrifuge tube.

34. Add 100-200 µL Elution Buffer heated to 65°C directly to the center of the HiBind® matrix.

35. Let sit at room temperature for 2 minutes.

36. Centrifuge at maximum speed for 1 minute.

37. Store DNA at -20°C.

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

E.Z.N.A.® Stool DNA Protocol for Large Volumes of Stool

The following protocol is designed when the targeting DNA is not distributed homogeneously in the stool sample. Using large volumes 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 at **1-800-832-8896** for more information.

Materials and Equipment to be Supplied by User:

- Centrifuge with adaptor for 15 mL or 50 mL centrifuge tubes capable of 4,000 x *g*
- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 mL and 2 mL microcentrifuge tubes
- 15 mL or 50 mL centrifuge tubes
- Water baths, heat blocks, or incubators capable of 65°C and 70°C
- Vortexer
- 100% ethanol
- Isopropanol
- Ice bath
- Optional: RNase A stock solution at 20 mg/mL
- Optional: Incubator capable of 95°C

Before Starting:

- Prepare ice bath
- Heat Elution Buffer to 65°C
- Set a water bath, heat block, or incubator to 70°C
- Prepare DNA Wash Buffer and VHB Buffer according to the Preparing Reagent section on Page 5
- Optional: for gram-positive bacteria set an incubator to 95°C

1. Add up to 2 g stool sample to a 15 mL or 50 mL centrifuge tube and place the tube on ice.

2. Add 10 volumes SLB Buffer. Vortex at maximum speed for 1 minute or until the stool sample is thoroughly homogenized.

   Note: For example, add 10 mL SLB Buffer to 1 g stool sample.

3. Add 1/10 volume DS Buffer and 20 µL Proteinase K Solution. Mix by inverting 10 times.

   Note: For example, if 10 mL SLB Buffer was used, add 1 mL DS Buffer.

4. Incubate at 70°C for 10 minutes.

5. Centrifuge at maximum speed (≥4,000 x *g*) for 15 minutes.

6. Transfer the supernatant to a new 15 mL or 50 mL centrifuge tube.

   Note: To make pipetting easier for viscous stool samples, cut the end of the pipet tips.

7. Add 1/3 volume SP2 Buffer. Vortex at maximum speed for 10 seconds.

8. Let sit on ice for 5 minutes.

9. Centrifuge at maximum speed (≥13,000 x *g*) for 10 minutes.

10. Transfer the cleared supernatant to a new 15 or 50 mL centrifuge tube.

11. Add 1 volume isopropanol. Invert the tube 10 times to mix.

12. Centrifuge at maximum speed for 10 minutes.

13. Discard the supernatant and invert the tube on a absorbent paper to drain the liquid drops.

14. Add 250 µL Elution Buffer. Vortex at maximum speed for 20 seconds.

15. Incubate at 70°C for 10-20 minutes. Vortex the sample twice during incubation.

Optional: If RNA-free DNA is required, add 10 µL RNase A. Vortex to mix thoroughly.

16. Add 200 µL HTR Reagent. Vortex at maximum speed for 10 seconds.

    Note: HTR Reagent must be thoroughly resuspended before use. Cut the end of a 1 mL tip to make it easier to pipet the HTR Reagent.

17. Let sit at room temperature for 2 minutes.

18. Centrifuge at maximum speed for 2 minutes.

19. Transfer 250 µL supernatant into a new 1.5 mL microcentrifuge tube.

20. Add 10 µL Proteinase K. Vortex to mix thoroughly.

21. Add 250 µL BL Buffer. Vortex at maximum speed for 10 seconds.

22. Incubate at 70°C for 5 minutes. Vortex the sample twice during incubation.

23. Centrifuge briefly to remove any liquid drops from the tube lid.

24. Add 250 µL 100% ethanol. Vortex at maximum speed for 10 seconds.

25. Centrifuge briefly to remove any liquid drops from the tube lid.

26. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

27. Transfer the entire sample from Step 13, including any precipitates that may have formed, to the HiBind® DNA Mini Column.

28. Centrifuge at maximum speed for 1 minute.

29. Discard the filtrate and collection tube.

30. Transfer the HiBind® DNA Mini Column to a new 2 mL Collection Tube.

31. Add 500 µL VHB Buffer.

    Note: VHB Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 5 for instructions.

32. Centrifuge at maximum speed for 30 seconds.

33. Discard the filtrate and reuse collection tube.

34. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with ethanol before use. Please see the Preparing Reagents section on Page 5 for instructions.

35. Centrifuge at maximum speed for 1 minute.

36. Discard the filtrate and reuse collection tube.

37. Repeat Steps 28-30 for a second DNA Wash Buffer wash step.

38. Centrifuge at maximum speed for 2 minutes to dry the column.

    Note: It is important to dry the column membrane before elution. Residual ethanol may interfere with downstream applications.

39. Transfer the column into a clean 1.5 mL microcentrifuge tube.

40. Add 200 µL Elution Buffer heated to 65°C directly to the center of the HiBind® matrix.

41. Let sit at room temperature for 2 minutes.

42. Centrifuge at maximum speed for 1 minute.

43. Store DNA at -20°C.

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

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
	Ethanol not added to the lysate before loading the column	Repeat the DNA isolation with a new sample
	No ethanol added to DNA Wash Buffer	Prepare DNA Wash Buffer with 100% ethanol
Problem	Cause	Solution
A_{260}/A_{280} ratio is high	RNA contamination	Treat the sample with RNase A according to the protocol
Problem	Cause	Solution
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 SLB Buffer thoroughly
	DNA washed off	Dilute DNA Wash Buffer with 100% ethanol prior to use (Page 5)
	Column matrix loses binding capacity during storage	Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x *g* for 30 seconds. Add 100 µL water to the columns and centrifuge at 10,000 x *g* for 30 seconds. Discard the filtrate.

Problem	Cause	Solution
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 eluted DNA before use 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 1:50 if necessary
	Ethanol residue in elute	Completely dry column before elution
Problem	Cause	Solution
Little or no supernatant after initial centrifuge step	Insufficient centrifugal force	Check the centrifugal force and increase the centrifugal time if necessary
Problem	Cause	Solution
Sample can not pass through the column	Clogged column	Check the centrifugal force and increase the time of centrifugation

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
HiBind® DNA Mini Columns (200 columns)	DNACOL-02
SP2 Buffer (60 mL)	PD073
DNA Wash Buffer (40 mL)	PDR044
Elution Buffer (100 mL)	PDR048
Proteinase K Solution	AC116
RNase A (400 µL)	AC117

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