OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Food DNA Kit

D4616-00	5 preps
D4616-01	50 preps

August 2016

For research use only.Not intended for diagnostic testing.

E.Z.N.A.® Food DNA Kit

Table of Contents

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Introduction

The E.Z.N.A.® Food DNA Kit allows rapid and reliable isolation of high-quality DNA from complex matrixes such as processed food, chocolate, cereals, and meat. Specific protocols exist for target host DNA for genetically modified organism (GMO) testing or bacterial DNA for pathogen/spoilation testing. Omega Bio-tek's MB1 Buffer allows for efficient homogenization of samples without foaming (commonly seen in lysis buffers containing detergents) resulting in higher yields.

The system combines the Omega Bio-tek's HiBind® DNA Mini Columns with RBB Buffer to eliminate PCR inhibiting compounds within the samples and elute highly concentrated DNA. The purified DNA can be used in PCR-based testing for GMO DNA. There are no organic extractions thus reducing plastic waste and hands-on time and multiple samples can be processed in parallel.

Kit Contents

Product Number	D4616-00	D4616-01
Purifications	5 preps	50 preps
HiBind® DNA Mini Columns	5	50
2 mL Collection Tubes	10	100
MB1 Buffer	5 mL	50 mL
MB2 Buffer	500 µL	5 mL
TBP Buffer	6 mL	60 mL
RBB Buffer	5 mL	50 mL
HBC Buffer	4 mL	25 mL
DNA Wash Buffer	1.5 mL	15 mL
Elution Buffer	2 mL	15 mL
Proteinase K Solution	150 µL	1.5 mL
User Manual	✓	✓

Storage and Stability

All of the E.Z.N.A.® Food DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows. Proteinase K Solution can be stored at room temperature for up to 6 months. For long-term storage, store Proteinase K Solution at 2-8°C. All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in some of the buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

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

Kit	100% Ethanol to be Added
D4616-00	6 mL
D4616-01	60 mL

2. Dilute HBC Buffer with 100% isopropanol follows and store at room temperature.

Kit	100% Isopropanol to be Added
D4616-00	1.6 mL
D4616-01	10.0 mL

E.Z.N.A.® Food DNA Kit Protocol for Host/GMO DNA

The following standard protocol is suitable for the isolation of DNA from up to 200 mg sample. Yields vary depending on source. The protocol can accomodate dried samples that absorb more liquid such as cereals, but additional MB1 Buffer and MB2 Buffer will need to be purchased separately. Please refer to the "Ordering Information" section on Page 18 or contact Customer Sevice, toll-free, at **1-800-832-8896**.

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 12,000 x *g*
- Incubator or heat block capable of 70°C
- 2 mL microcentrifuge tubes
- Nuclease-free 1.5 mL microcentrifuge tubes for DNA storage
- Vortexer
- 100% ethanol
- 100% isopropanol
- Grinding beads: 3-4 mm steel or ceramic beads
- Centrifuge tubes or vials for sample grinding
- Optional: Mixer mill such as a SPEX CertiPrep Geno/Grinder® 2010 or Qiagen TissueLyser, Eppendorf MixMate

Before Starting:

- Prepare HBC Buffer and DNA Wash Buffer according to the "Preparing Reagents" section on Page 4.
- Set an incubator to 60°C.
- Heat Elution Buffer to 70°C.

1. Add up to 200 mg sample and 2 grinding beads (not provided) to a centrifuge tube/vial (not provided) which can be used for grinding the sample in the subsequent steps.

2. Add 700 µL MB1 Buffer.

   Note: The volume of MB1 Buffer may need to be adjusted depending on the sample type. For samples which absorb more liquid (cereals, dried powder), use 950 µL MB1 Buffer. Additional buffer can be purchased separately.

3. Vortex at maximum speed for 3-5 minutes to lyse and homogenize the samples. For best results, a mixer mill, such as Spex CertiPrep Geno/Grinder® 2010 or Qiagen Tissuelyser, should be used.

4. Centrifuge at 1,000-2,000 x *g* for 15 seconds at room temperature.

5. Add 84 µL MB2 Buffer and 20 µL Proteinase K Solution to the sample.

   Note: If more MB1 Buffer is used in Step2, then adjust MB2 Buffer accordingly. Additional buffer can be purchased separately. The volume of Proteinase K Solution does not need to be changed.

6. Vortex for 60 seconds to mix thoroughly.

7. Incubate at 60°C for 20 minutes. Mix once during incubation.

8. Centrifuge at maximum speed (≥12,000 x *g*) for 5 minutes.

9. Transfer the cleared supernatant to a 2 mL microcentrifuge tube (not provided).

   Note: Do not transfer any debris as it can reduce yield and purity.

10. Add 2 volumes RBB Buffer. Vortex to mix thoroughly.

    Note: 2 volumes RBB Buffer refers to the amount of cleared supernatant recovered during Step 9. If 400 µL is recovered, add 800 µL RBB Buffer.

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

12. Transfer 700 µL sample from Step 10 to the HiBind® DNA Mini Column.

13. Centrifuge at maximum speed for 1 minute.

14. Discard the filtrate and reuse the collection tube.

15. Repeat Steps 12-14 until all the lysate from Step 10 has been passed through the HiBind® DNA Mini Column.

16. Add 600 µL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 4 for instructions.

17. Centrifuge at maximum speed for 30 seconds.

18. Discard the filtrate and collection tube.

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

20. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 4 for instructions.

21. Centrifuge at maximum speed for 30 seconds.

22. Discard the filtrate and reuse the collection tube.

23. Repeat Steps 20-22 for a second DNA Wash Buffer wash step.

24. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

25. Transfer the HiBind® DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube (not provided).

26. Add 50-100 µL Elution Buffer heated to 70°C.

27. Let sit at room temperature for 2 minutes.

28. Centrifuge at maximum speed for 1 minute.

29. Transfer the filtrate from Step 28 to the HiBind® DNA Mini Column.

30. Let sit at room temperature for 2 minutes.

31. Centrifuge at maximum speed for 1 minute.

32. Store eluted DNA at -20°C.

E.Z.N.A.® Food DNA Kit Protocol for Pathogens

The following standard protocol is suitable for the isolation of DNA from up to 200 mg sample. Yields vary depending on source. The protocol can accomodate dried samples that absorb more liquid such as cereals, but additional MB1 Buffer and MB2 Buffer will need to be purchased separately. Please refer to the "Ordering Information" section on Page 18 or contact Customer Sevice, toll-free, at **1-800-832-8896**.

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 12,000 x *g*
- Incubator or heat block capable of 70°C
- 2 mL microcentrifuge tubes
- Nuclease-free 1.5 mL microcentrifuge tubes for DNA storage
- Vortexer
- 100% ethanol
- 100% isopropanol
- Grinding beads: 3-4 mm steel or ceramic beads
- Grinding beads: 0.1 mm beads in a 2 mL microcentrifuge tube - Recommend Omni International Micro-Organism Lysing Mix (Cat# 19-621)
- Optional: mixer mill such as a SPEX CertiPrep Geno/Grinder® 2010 or Qiagen TissueLyser, Eppendorf MixMate

Before Starting:

- Prepare HBC Buffer and DNA Wash Buffer according to the "Preparing Reagents" section on Page 4.
- Set an incubator or heat block to 60°C.
- Heat Elution Buffer to 70°C.

1. Add up to 200 mg sample and 2 grinding beads into a 2 mL microcentrifuge tube containing 0.1 mm glass beads (not provided). If an overnight pre-culture has been used, centrifuge an aliquot of the culture and use the pellet for the sample.

   Note: We recommend Omni International Micro-Organism Lysing Mix for the 0.1 mm glass beads.

2. Add 700 µL MB1 Buffer.

   Note: The volume of MB1 Buffer may need to be adjusted depending on sample type. For samples which absorb more liquid (cereals, dried powder), use 950 µL MB1 Buffer. Additional buffer can be purchased separately.

3. Vortex at maximum speed for 3-5 minutes to lyse and homogenize the samples. For best results, a Mixer Mill, such as Spex CertiPrep Geno/Grinder® 2010 or Qiagen Tissuelyser, should be used.

4. Centrifuge at 1,000-2,000 x *g* for 15 seconds at room temperature.

5. Add 84 µL MB2 Buffer and 20 µL Proteinase K Solution to the sample.

   Note: If more MB1 Buffer is used in Step 2, then adjust MB2 Buffer accordingly. Additional buffer can be purchased separately. The volume of Proteinase K Solution does not need to be changed.

6. Vortex for 60 seconds to mix thoroughly.

7. Incubate at 60°C for 20 minutes. Mix once during incubation.

8. Centrifuge at maximum speed (≥12,000 x *g*) for 5 minutes.

9. Transfer the cleared supernatant to a 2 mL microcentrifuge tube (not provided).

   Note: Do not transfer any debris as it can reduce yield and purity.

10. Add 2 volumes RBB Buffer. Vortex to mix thoroughly.

    Note: 2 volumes RBB Buffer refers to the amount of cleared supernatant recovered during Step 9. If 400 µL is recovered, add 800 µL RBB Buffer.

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

12. Transfer 700 µL sample from Step 10 to the HiBind® DNA Mini Column.

13. Centrifuge at maximum speed for 1 minute.

14. Discard the filtrate and reuse the collection tube.

15. Repeat Steps 12-14 until all the lysate from Step 10 has been passed through the HiBind® DNA Mini Column.

16. Add 600 µL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 4 for instructions.

17. Centrifuge at maximum speed for 30 seconds.

18. Discard the filtrate and collection tube.

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

20. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 4 for instructions.

21. Centrifuge at maximum speed for 30 seconds.

22. Discard the filtrate and reuse the collection tube.

23. Repeat Steps 20-22 for a second DNA Wash Buffer wash step.

24. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications**.**

25. Transfer the HiBind® DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube (not provided).

26. Add 50-100 µL Elution Buffer heated to 70°C.

27. Let sit at room temperature for 2 minutes.

28. Centrifuge at maximum speed for 1 minute.

29. Transfer the filtrate from Step 28 to the HiBind® DNA Mini Column.

30. Let sit at room temperature for 2 minutes.

31. Centrifuge at maximum speed for 1 minute.

32. Store eluted DNA at -20°C.

E.Z.N.A.® Food DNA Kit Protocol for Milk

Materials and Equipment to be Supplied by User:

- Centrifuge capable of at least 12,000 x *g*
- Incubator capable of 70°C
- 2 mL microentrifuge tubes
- Nuclease-free 1.5 mL microcentrifuge tubes for DNA storage
- Vortexer
- 100% ethanol
- 100% isopropanol
- Grinding beads: 0.1 mm beads in a 2 mL microcentrifuge tube - Recommend Omni International Micro-Organism Lysing Mix (Cat# 19-621)
- Lysozyme (10 mg/mL)
- Optional: mixer mill such as a SPEX CertiPrep Geno/Grinder® 2010 or Qiagen TissueLyser, Eppendorf MixMate

Before Starting:

- Prepare HBC Buffer and DNA Wash Buffer according to the "Preparing Reagents" section on Page 4.
- Set an incubator or heat block to 37°C.
- Set an incubator or heat block to 60°C.
- Heat Elution Buffer to 70°C.

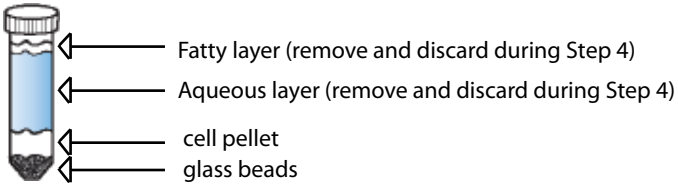
1. Add up to 0.5 mL milk sample to a 2 mL microcentrufuge tube containing 0.1 mm glass beads and then add 1 mL TBP Buffer.

   Note: We recommend Omni International Micro-Organism Lysing Mix for the 0.1 mm glass beads.

2. Invert 10 times to mix.

3. Centrifuge at 3,000 x *g* for 10 minutes at room temperature.

4. Remove and discard the aqueous and fatty layer.



5. Add 400 µL MB1 Buffer.

6. Vortex at maximum speed for 3-5 minutes to lyse and homogenize the samples. For best results, a mixer mill, such as Spex CertiPrep Geno/Grinder® 2010 or Qiagen Tissuelyser, should be used.

7. Add 20 µL lysozyme (10 mg/mL). Invert 10 times to mix.

8. Incubate at 37°C for 10 minutes.

9. Centrifuge at 1,000-2,000 x *g* for 15 seconds at room temperature.

10. Add 50 µL MB2 Buffer and 20 µL Proteinase K Solution.

11. Vortex for 60 seconds to mix thoroughly.

12. Incubate for 1 hour at 60°C.

13. Centrifuge at maximum speed (≥12,000 x *g*) for 5 minutes.

14. Transfer the cleared supernatant to a 2 mL microcentrifuge tube (not provided).

    Note: Do not transfer any debris as it can reduce yield and purity.

15. Add 2 volumes RBB Buffer. Vortex to mix thoroughly.

    Note: 2 volumes RBB Buffer refers to the amount of cleared supernatant recovered during Step 14. If 400 µL is recovered, add 800 µL RBB Buffer.

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

17. Transfer 700 µL sample from Step 15 to the HiBind® DNA Mini Column.

18. Centrifuge at maximum speed for 1 minute.

19. Discard the filtrate and reuse the collection tube.

20. Repeat Steps 17-19 until all the lysate from Step 15 has been passed thru the HiBind® DNA Mini Column.

21. Add 600 µL HBC Buffer.

    Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 4 for instructions.

22. Centrifuge at maximum speed for 30 seconds.

23. Discard the filtrate and collection tube.

24. Insert the HiBind® DNA Mini Column into a new 2 mL Collection Tube.

25. Add 700 µL DNA Wash Buffer.

    Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 4 for instructions.

26. Centrifuge at maximum speed for 30 seconds.

27. Discard the filtrate and reuse the collection tube.

28. Repeat Steps 25-27 for a second DNA Wash Buffer wash step.

29. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column.

    Note: This step is critical for removal of trace ethanol that may interfere with downstream applications.

30. Transfer the HiBind® DNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube (not provided).

31. Add 50-100 µL Elution Buffer heated to 70°C.

32. Let sit at room temperature for 2 minutes.

33. Centrifuge at maximum speed for 1 minute.

34. Transfer the filtrate from Step 33 to the HiBind® DNA Mini Column.

35. Let sit at room temperature for 2 minutes.

36. Centrifuge at maximum speed for 1 minute.

37. Store eluted DNA at -20°C.

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	Salt contamination	• Perform a second wash with HBC Buffer. • Centrifuge at 13,000 x *g* for 5 minutes during HiBind DNA Mini Column drying step • Make sure DNA Wash Buffer was prepared using ethanol and not isopropanol
A_{260}/A_{280} ratio is high	RNA contamination	The protocol does not remove RNA. If desired, add 5 µL RNase A (25 mg/mL) after lysate is cleared and before binding buffers are added. Let sit at room temperature for 5 minutes.
Low DNA Yield or no DNA Yield	Poor homogenization of sample	Repeat the DNA isolation with a new sample, be sure to mix the sample with MB1 Buffer thoroughly. Use a commercial homogenizer if possible.
	DNA washed off	Make sure HBC Buffer is mixed with isopropanol and DNA Wash Buffer is mixed with ethanol.
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 DNA elute used in the downstream application if possible.
	Non-specific bands in downstream PCR	Use hot-start Taq polymerase mixture.
Problems in downstream applications	Inhibitory substance in the eluted DNA	Check the A_{260}/A_{230} ratio. Dilute the elute to 1:50 if necessary.

Ordering Information

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

Product	Part Number
MB1 Buffer, 100 mL	MB1-100
DNA Wash Buffer, 100 mL	PS010
Elution Buffer, 100 mL	PDR048
RNase A (25 mg/mL) 400 µL	AC117