



E.Z.N.A.[®] Bacterial DNA Kit

D3350-00	5 preps
D3350-01	50 preps
D3350-02	200 preps

Manual Date: August 2019
Revision Number: v7.1

For Research Use Only

E.Z.N.A.® Bacterial DNA Kit

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Introduction and Overview

Introduction

The E.Z.N.A.® Bacterial DNA Kit allows rapid and reliable isolation of high-quality total cellular DNA from a wide variety of gram-positive and gram-negative bacterial species. Up to 1×10^9 bacterial cells can be processed. The system combines the reversible nucleic acid-binding properties of Omega Bio-tek's HiBind® matrix with the speed and versatility of spin column technology to yield up to 15-30 µg of DNA with an A_{260}/A_{280} ratio of 1.7-1.9. Purified DNA is suitable for PCR, restriction enzyme digestion, and hybridization applications. There are no organic extractions which reduces plastic waste and user time allowing multiple samples to be processed in parallel.

E.Z.N.A.® Bacterial DNA Kit will isolate all cellular DNA, including plasmid DNA.

Each HiBind® DNA Mini Column can bind approximately 100 µg genomic DNA. Using greater than 1×10^9 bacterial cells is not recommended.

Overview

If using the E.Z.N.A.® Bacterial DNA Kit for the first time, please read this booklet to become familiar with the procedures. Bacterial cells are grown to log-phase and harvested. The bacterial cell wall is removed by lysozyme digestion followed by Proteinase K digestion. Following lysis, binding conditions are adjusted and the sample is applied to a HiBind® DNA spin column. Three rapid wash steps remove trace salts and protein contaminants and DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

New in this Edition:

- April 2017: VAC-08, Omega's vacuum manifold had been discontinued and is no longer available for purchase. Compatible vacuum manifolds are listed on Page 5.
- January 2016: BTL Buffer has been renamed TL Buffer. This is a name change only. The buffer formulation has not changed. BDL Buffer has been renamed BL Buffer. This is a name change only. The buffer formulation has not changed.
- June 2014: Glass Beads are now called Glass Beads S. This is a name change only. The component has not changed.
- May 2013: HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.
- April 2013: 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.

Kit Contents

Product	D3350-00	D3350-01	D3350-02
Purifications	5 preps	50 preps	200 preps
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
TL Buffer	1.5 mL	20 mL	50 mL
BL Buffer	2 mL	20 mL	50 mL
HBC Buffer	5 mL	25 mL	80 mL
DNA Wash Buffer	2.5 mL	25 mL	3 x 25 mL
Elution Buffer	15 mL	30 mL	60 mL
Glass Beads S	150 mg	2 g	8 g
Proteinase K Solution	150 µL	1.5 mL	6.0 mL
RNase A	30 µL	275 µL	1.1 mL
Lysozyme	5 mg	50 mg	4 x 50 mg
User Manual	✓	✓	✓

Storage and Stability

All E.Z.N.A.® Bacterial 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 12 months. For long-term storage (>12 months), store at 2-8°C. Once reconstituted in buffer, Lysozyme must be stored at -20°C. Store RNase A at 2-8°C. Store all other components at room temperature. Check buffers for precipitates before use. Redissolve any precipitates by warming to 37°C.

Preparing Reagents

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

Kit	100% Isopropanol to be Added
D3350-00	2 mL
D3350-01	10 mL
D3350-02	32 mL

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

Kit	100% Ethanol To Be Added
D3350-00	10 mL
D3350-01	100 mL
D3350-02	100 mL to each bottle

3. Dissolve Lysozyme with Elution Buffer as follows and store at -20°C.

Kit	Elution Buffer To Be Added
D3350-00	100 μ L
D3350-01	1 mL
D3350-02	1 mL to each vial

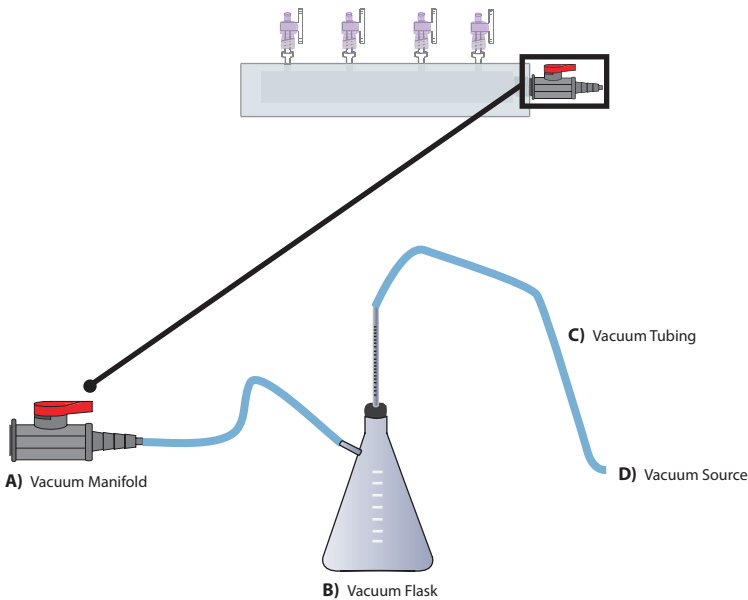
Guidelines for Vacuum Manifold

The following is required for use with the Vacuum Protocol:

- A) Vacuum Manifold
 - Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma Aldrich VM20, Promega Vacman®, or manifold with standard Luer connector
- B) Vacuum Flask
- C) Vacuum Tubing
- D) Vacuum Source (review tables below for pressure settings)

Conversion from millibars:	Multiply by:
millimeters of mercury (mmHg)	0.75
kilopascals (kPa)	0.1
inches of mercury (inHg)	0.0295
Torrs (Torr)	0.75
atmospheres (atm)	0.000987
pounds per square inch (psi)	0.0145

Illustrated Vacuum Setup:



E.Z.N.A.[®] Bacterial DNA Protocol

E.Z.N.A.[®] Bacterial DNA Kit - Centrifugation Protocol

This method allows genomic bacterial isolation from up to 3 mL LB culture.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of 13,000 x *g*
- Centrifuge to pellet culture capable of 4,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water bath capable of 37°C
- Shaking water bath capable of 55°C
- Incubator or water bath capable of 65°C
- Vortexer
- 100% ethanol
- 100% isopropanol
- TE Buffer

Before Starting:

- Prepare HBC Buffer, DNA Wash Buffer, and Lysozyme, as instructed in the "Preparing Reagents" section on Page 4.
 - Set a water bath to 37°C.
 - Set a shaking water bath to 55°C.
 - Set an incubator or water bath to 65°C.
 - Heat Elution Buffer to 65°C.
-
1. Culture bacteria in LB media to log-phase. (Overnight culture can be used in many cases.)
 2. Centrifuge no more than 3 mL culture or 1×10^9 cells at 4,000 x *g* for 10 minutes at room temperature.
 3. Aspirate and discard the media.
 4. Add 100 μ L TE Buffer. Vortex to completely resuspend the pellet.

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5. Add 10 μ L Lysozyme.

Note: Lysozyme must be resuspended with Elution Buffer before use. Please see Page 4 for instructions.

6. Incubate at 37°C for 10 minutes.

Note: The amount of enzyme required and/or the length of incubation may need to be modified depending on the bacterial strain used. Complete digestion of the cell wall is essential for efficient lysis. Longer incubation time may yield better results.

Optional: Follow the short protocol below for difficult to lyse bacteria.

1. Add 25 mg Glass Beads S to a 1.5 mL microcentrifuge tube (not provided).
2. Add sample to the Glass Beads S.
3. Vortex at maximum speed for 5 minutes.
4. Let sample stand to allow the beads to settle.
5. Transfer supernatant to a new 1.5 mL microcentrifuge tube.

7. Add 100 μ L TL Buffer and 20 μ L Proteinase K Solution. Vortex to mix thoroughly.

8. Incubate at 55°C in a shaking water bath.

Note: Usually no more than 1 hour is required for bacterial lysis. If a shaking water bath is not available, incubate the samples and shake or briefly vortex every 20-30 minutes.

9. Add 5 μ L RNase A. Invert tube several times to mix.

10. Let sit at room temperature for 5 minutes.

11. Centrifuge at 10,000 $\times g$ for 2 minutes to pellet any undigested material.

12. Transfer the supernatant to a new 1.5 mL microcentrifuge tube (not provided). Do not disturb the pellet.

13. Add 220 μ L BL Buffer. Vortex to mix thoroughly.

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14. Incubate at 65°C for 10 minutes.

Note: A wispy precipitate may form upon addition of BL Buffer; it does not interfere with DNA recovery.

15. Add 220 µL 100% ethanol. Vortex for 20 seconds at maximum speed to mix thoroughly.

Note: If any precipitate can be seen at this point, break the precipitate by pipetting up and down 10 times.

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

17. Transfer the entire sample to the HiBind® DNA Mini Column, including any precipitate that may have formed.

18. Centrifuge at 10,000 x *g* for 1 minute.

19. Discard the filtrate and the collection tube.

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

21. Add 500 µL HBC Buffer.

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

22. Centrifuge at 10,000 x *g* for 1 minute.

23. Discard the filtrate and reuse the collection tube.

24. Add 700 µL DNA Wash Buffer.

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

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25. Centrifuge at 10,000 x *g* for 1 minute.
26. Discard the filtrate and reuse the collection tube.
27. Repeat Steps 24-26 for a second DNA Wash Buffer wash step.
28. Centrifuge the empty HiBind® DNA Mini Column at maximum speed ($\geq 13,000 \times g$) for 2 minutes to dry the column.

Note: This step is critical for removal of trace ethanol that may interfere with downstream applications.
29. Insert the HiBind® DNA Mini Column into a new nuclease-free 1.5 mL microcentrifuge tube.
30. Add 50-100 μ L Elution Buffer heated 65°C.

Note: Make sure to add the Elution Buffer to the center of the HiBind® matrix. Each 50-100 μ L elution typically yields 60-70% of the DNA bound to the HiBind® matrix. Two elutions generally yield ~90%. However, increasing elution volume reduces the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 50 μ L Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 μ L greatly reduce yields.
31. Let sit for 3 to 5 minutes at room temperature.

Note: Yields may be increased by incubating the column at 65°C rather than at room temperature.
32. Centrifuge at 10,000 x *g* for 1 minute to elute the DNA.
33. Repeat Steps 30-32 for a second elution step.
34. Store eluted DNA at -20°C.

E.Z.N.A.[®] Bacterial DNA Protocol

E.Z.N.A.[®] Bacterial DNA Kit - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Vacuum manifold with standard Luer adaptor
- Microcentrifuge capable of 13,000 x g
- Centrifuge to pellet culture capable of 4,000 x g
- Nuclease-free 1.5 mL microcentrifuge tubes
- Water bath capable of 37°C
- Shaking water bath capable of 55°C
- Incubator or water bath capable of 65°C
- Vortexer
- 100% ethanol
- 100% isopropanol
- TE Buffer

Before Starting:

- Prepare HBC Buffer, DNA Wash Buffer, and Lysozyme, as instructed in the "Preparing Reagents" section on Page 4.
- Set a water bath to 37°C.
- Set a shaking water bath to 55°C.
- Set an incubator or water bath to 65°C.
- Heat Elution Buffer to 65°C.

1. Culture bacteria in LB media to log-phase. (Overnight culture can be used in many cases.)
2. Centrifuge no more than 3 mL culture or 1×10^9 cells at 4,000 x g for 10 minutes at room temperature.
3. Aspirate and discard the media.
4. Add 100 µL TE Buffer. Vortex to completely resuspend the pellet.
5. Add 10 µL Lysozyme.

Note: Lysozyme must be resuspended with Elution Buffer before use. Please see Page 4 for instructions.

E.Z.N.A.® Bacterial DNA Protocol

6. Incubate at 37°C for 10 minutes.

Note: The amount of enzyme required and/or the length of incubation may need to be modified depending on the bacterial strain used. Complete digestion of the cell wall is essential for efficient lysis. Longer incubation time may yield better results.

Optional: Follow the short protocol below for difficult to lyse bacteria.

1. Add 25 mg Glass Beads S to 1.5 mL microcentrifuge tube (not provided).
 2. Add sample to the Glass Beads S.
 3. Vortex at maximum speed for 5 minutes.
 4. Let sample stand to allow the beads to settle.
 5. Transfer supernatant to a new 1.5 mL microcentrifuge tube.
7. Add 100 µL TL Buffer and 20 µL Proteinase K Solution. Vortex to mix thoroughly.
 8. Incubate at 55°C in a shaking water bath.

Note: Usually no more than 1 hour is required for bacterial lysis. If a shaking water bath is not available, incubate the samples and shake or briefly vortex every 20-30 minutes.

9. Add 5 µL RNase A. Invert tube several times to mix.
10. Let sit at room temperature for 5 minutes.
11. Centrifuge at 10,000 x g for 2 minutes to pellet any undigested material.
12. Transfer the supernatant to a new 1.5 mL microcentrifuge tube (not provided). Do not disturb the pellet.
13. Add 220 µL BL Buffer. Vortex to mix thoroughly.
14. Incubate at 65°C for 10 minutes.

Note: A wispy precipitate may form upon addition of BL Buffer; it does not interfere with DNA recovery.

E.Z.N.A.® Bacterial DNA Protocol

15. Add 220 µL 100% ethanol. Vortex for 20 seconds at maximum speed to mix thoroughly.

Note: If any precipitate can be seen at this point, break the precipitate by pipetting up and down 10 times.

16. Prepare the vacuum manifold according to manufacturer's instructions and connect the HiBind® DNA Mini Column to the manifold.
17. Transfer the entire sample to the HiBind® DNA Mini Column, including any precipitate that may have formed.
18. Switch on the vacuum source to draw the sample through the column.
19. Turn off the vacuum.
20. Add 500 µL HBC Buffer.

Note: HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 4 for instructions.
21. Switch on the vacuum source to draw the HBC Buffer through the column.
22. Turn off the vacuum.
23. Add 700 µL DNA Wash Buffer.

Note: DNA Wash Buffer must be diluted with 100% ethanol before use. Please see Page 4 for instructions.
24. Switch on the vacuum source to draw the DNA Wash Buffer through the column.
25. Turn off the vacuum.
26. Repeat Steps 23-25 for a second DNA Wash step.

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27. Remove the HiBind® DNA Mini Column from the vacuum manifold and transfer to a new 2 mL Collection Tube (provided).

28. Centrifuge at maximum speed ($\geq 13,000 \times g$) for 3 minutes to completely dry the membrane.

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

29. Insert the HiBind® DNA Mini Column into a new nuclease-free 1.5 mL microcentrifuge tube.

30. Add 50-100 μ L Elution Buffer heated to 65°C.

Note: Make sure to add the Elution Buffer to the center of the HiBind® matrix. Each 50-100 μ L elution typically yields 60-70% of the DNA bound to the HiBind® matrix. Two elutions generally yield ~90%. However, increasing elution volume reduces the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using 50 μ L Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 μ L greatly reduce yields.

31. Let sit for 3 to 5 minutes at room temperature.

Note: Yields may be increased by incubating the column at 65°C rather than at room temperature.

32. Centrifuge at 10,000 $\times g$ for 1 minute to elute the DNA.

33. Repeat Steps 30-32 for a second elution step.

34. 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
Clogged column	Incomplete lysis	Add the correct volume of TL Buffer and incubate at 55°C to obtain complete lysis. It may be necessary to extend incubation time to 1 hour.
	Too much sample	Do not use greater than 3 mL culture at OD ₆₀₀ 10 or 1 x 10 ⁹ cell per mini column. For larger volumes, divide sample into multiple tubes.
	Incomplete removal of cell wall	Add more Lysozyme or extend the incubation time. It may be necessary to increase incubation by 15 minutes.
Problem	Cause	Solution
Low DNA yield	Clogged column	See above.
	Poor elution	Repeat elution or increase elution volume (see note on Page 9). Incubation of column at 65°C for 5 minutes after addition of Elution Buffer may increase yield.
	Improper washing	DNA Wash Buffer must be diluted with 100% ethanol.
	Column needs priming	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.

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PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.

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
BL Buffer, 100 mL	PD062
TL Buffer, 200 mL	PD061
DNA Wash Buffer, 100 mL	PS010
Elution Buffer, 100 mL	PDR048
Proteinase K Solution, 10 mL	AC116

Notes:

For more purification solutions, visit www.omegabiotek.com

AVAILABLE FORMATS



Spin Columns



96-Well
Silica Plates



Mag Beads

SAMPLE TYPES



Blood / Plasma



Plasmid



Cultured Cells



Plant & Soil



NGS Clean Up



Tissue



FFPE




Fecal Matter



BIO-TEK

innovations in nucleic acid isolation

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