omega BIO-TEK

innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Bacterial DNA Kit

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

Manual Date: May 2013

For Research Use Only

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E.Z.N.A.® Bacterial DNA Kit

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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 x 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 x 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:

- HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.
- Equilibration Buffer can be replaced with 3M NaOH provided by the user.

Kit Contents

Product	D3350-00	D3350-01	D3350-02
Purification	5 preps	50 preps	200 preps
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
BTL Buffer	1.5 mL	20 mL	50 mL
BDL Buffer	2 mL	20 mL	50 mL
HBC Buffer	3 mL	25 mL	80 mL
DNA Wash Buffer	2 mL	15 mL	3 x 20 mL
Glass Beads	150 mg	2 g	8 g
Elution Buffer	5 mL	15 mL	60 mL
Lysozyme	5 mg	50 mg	4 x 50 mg
Proteinase K Solution	140 µL	1.4 mL	4 x 1.4 mL
RNase A	30 µL	275 µL	1.1 mL
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Storage and Stability

All E.Z.N.A.® Bacterial DNA Kit components are guaranteed for at least 24 months from the date of purchase when stored as follows. Proteinase K Solution can be stored at room temperature for 24 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

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

Kit	100% Ethanol To Be Added
D3350-00	8 mL
D3350-01	60 mL
D3350-02	80 mL to each bottle

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

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

- Dilute HBC Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D3350-00	1.2 mL
D3350-01	10 mL
D3350-02	32 mL

Guidelines for Vacuum Manifold

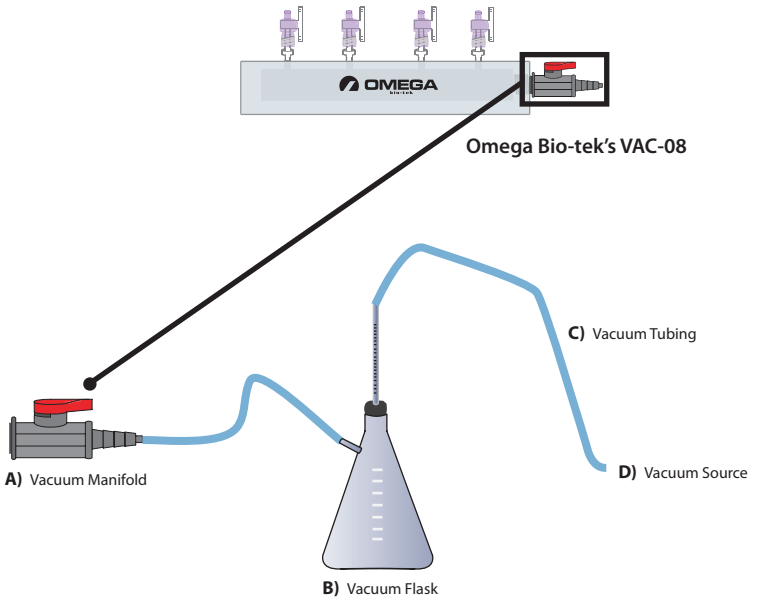
The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-08)
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)

Manifold	Recommended Pressure (mbar)
VAC-08	-200 to -600

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 - Centrifugation Protocol

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

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge
- 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
- 100% ethanol
- Isopropanol
- TE Buffer
- Vortexer

Before Starting:

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

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 to 1.5 mL microcentrifuge tube.
2. Add sample to the glass beads.
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 BTL 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. Incubate 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. Do not disturb the pellet.

13. Add 220 µL BDL Buffer. Vortex to mix.

14. Incubate at 65°C for 10 minutes.

    Note: A wispy precipitate may form upon addition of BDL 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 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.

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 (≥10,000 x *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 µL Elution Buffer heated 65°C to the HiBind® DNA Mini Column.

    Note: Make sure to add the Elution Buffer to the center of the HiBind® matrix. Each 50-100 µL 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 µL Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µL 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 - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Tabletop microcentrifuge
- 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
- 100% ethanol
- Isopropanol
- TE Buffer
- Vortexer
- Vacuum manifold with standard Luer adaptor

Before Starting:

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

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 to 1.5 mL microcentrifuge tube.
2. Add sample to the glass beads.
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 BTL 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. Incubate 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. Do not disturb the pellet.

13. Add 220 µL BDL Buffer. Vortex to mix.

14. Incubate at 65°C for 10 minutes.

    Note: A wispy precipitate may form upon addition of BDL 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. 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 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 isopropanol before use. Please see Page 4 for instructions.

21. Switch on vacuum source to draw the HBC Buffer through the column.

22. Turn off the vacuum.

23. Add 700 µL DNA Wash Buffer to the HiBind® DNA Mini Column.

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

24. Switch on 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.

27. Remove the column from the vacuum manifold and transfer to a new 2 ml collection tube provided with the kit.

28. Centrifuge at maximum speed (≥10,000 x g) for 2 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 µL Elution Buffer heated to 65°C to the HiBind® DNA Mini Column.

    Note: Make sure to add the Elution Buffer to the center of the HiBind® matrix. Each 50-100 µL 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 µL Elution Buffer (which slightly reduces overall DNA yield). Volumes lower than 50 µL 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.

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 BTL 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 spin 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.

Ordering Information

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

Product	Part Number
BDL Buffer (100 mL)	PD064
BTL Buffer (200 mL)	PD065
DNA Wash Buffer (100 mL)	PS010
Elution Buffer (100 mL)	PDR048

Notes:

For more purification solutions, visit **www.omegabiotek.com**

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Mag Beads

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Plasmid


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