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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 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 approximately 15-30 µg of DNA with an A_{260}/A_{280} ratio of 1.7-1.9. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions, thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

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

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. Two rapid wash steps remove trace salts and protein contaminants, and finally 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.

Storage and Stability

All components of the E.Z.N.A.® Bacterial DNA Kit, except the Proteinase K, RNase A and Lysozyme can be stored at 22°C-25°C and are guaranteed for at least 24 months from the date of purchase. Once reconstituted in water, Proteinase K and lysozyme must be stored at -20°C. Store RNase A at -20°C. Under cool ambient conditions, a precipitate may form in the Buffer BDL/BTL. In case of such an event, heat the bottle at 37°C to dissolve. Store Buffer BDL/BTL at room temperature.

Binding Capacity

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

Kit Contents

Product	D3350-00	D3350-01	D3350-02
Purification times	5 Preps	50 Preps	200 Preps
HiBind® DNA Mini Columns	5	50	200
2 ml Collection Tubes	10	100	400
Buffer BTL	1.5 ml	20 ml	50 ml
Buffer BDL	2 ml	20 ml	50 ml
Buffer HB	3 ml	30 ml	110 ml
DNA Wash Buffer concentrate	2 ml	20 ml	3 x 20 ml
Glass Powder	200 mg	2.0 g	8.0 g
Elution Buffer	1 ml	10 ml	40 ml
Lysozyme	5 mg	50 mg	4 x 50mg
Proteinase K	2.0 mg	19 mg	78 mg
Protease Storage Buffer	200 µl	1.8 ml	6 ml
RNase A	30µl	275µl	1.1 ml
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Buffer BDL contains a chaotropic salt. Use gloves and protective eyeware when handling this solution.

Materials to Be Provided by User

- Tabletop microcentrifuge and nuclease-free 1.5 ml tubes
- Water bath set to 30°C
- Shaking water bath set to 55°C
- Incubator or waterbath set to 65°C
- Absolute ethanol (96%-100%) - Do not use other alcohols

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.® Bacterial DNA Kit procedure.
- Prepare a stock solution of Proteinase K (provided) as follows and **aliquot into adequate portions. Store aliquots at -20°C.**

D3350-00	dissolve with 130 µl of Protease Storage Buffer
D3350-01	dissolve with 1.30 ml of Protease Storage Buffer
D3350-02	dissolve with 5.1 ml of Protease Storage Buffer

- **Prepare a lysozyme stock solution at 50 mg/ml and aliquot into adequate portions. Store each aliquot at -20°C and thaw before use**. Each sample will require 20 µl of this solution.

D3350-00	dissolve with 100 µl of Elution Buffer
D3350-01	dissolve with 1 ml of Elution Buffer
D3350-02	dissolve with 1 ml of Elution Buffer for each tube

- Equilibrate Elution Buffer provided to 65°C.
- Dilute DNA Wash Buffer Concentrate with ethanol as follows and store at room temperature:

D3350-00	Add 8 ml absolute (96%-100%) ethanol
D3350-01	Add 80 ml absolute (96%-100%) ethanol
D3350-02	Add 80 ml absolute (96%-100%) ethanol per bottle
Store the diluted DNA Wash Buffer at room temperature.	

- ***Carry out all of centrifugation step at room temperature.***

E.Z.N.A.™ Bacterial DNA Spin Protocol

1. This method allows genomic bacterial isolation from up to 3 ml LB culture. **Grow Bacteria in LB media to log phase**. (Overnight culture can be used in many cases.)

2. **Harvest no more than 3 ml culture** by centrifugation at 4,000 x g for 10 min at room temperature.

3. Remove medium completely and **resuspend cells in 180 µl TE buffer. Add 20 µl of 50 mg/ml lysozyme solution**, Incubate at 30°C for 10 min.

   Note: The amount of enzyme required and/or the incubation time 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 might yield better results.

4. Pellet digested cell by centrifugation at 5,000 x g for 5 min at room temperature. Discard the supernatant and add **200 µl Buffer BTL. Vortex to resuspend cells.**

5. **Optional: For complete digestion of bacterial cell wall especially of Gram-Positive bacteria, add 25-40 mg Glass Powder and vortex at maxi speed for 5 minutes.** Let it stand to allow the beads to settle. Transfer supernatant to a new 1.5 ml centrifuge tube (not supplied).

6. **Add 25 µl Proteinase K solution and vortex to mix well**. Incubate at 55°C in a shaking water bath to effect complete lysis. Usually no more than 1 h is required for bacterial lysis. If no shaking waterbath is available, incubate and shake or briefly vortex the samples every 20-30 minutes.

7. Add 5 µl RNase A to samples and invert tube several times to mix. Incubate at room temperature for 10-30 minutes.

8. (Optional) **Centrifuge at 10,000 x g for 5 min to pellet insoluble debris**. Carefully aspirate the supernatant and transfer to a sterile micro-centrifuge tube leaving behind any insoluble pellet.

9. **Add 220 µl Buffer BDL and shake or briefly vortex to mix. Incubate at 65°C for 10 minutes.** A wispy precipitate may form upon addition of Buffer BDL; it does not interfere with DNA recovery.

10. **Add 220 µl absolute ethanol (room temperature, 96-100%) and mix thoroughly by vortexing at maxi speed for 20 seconds.** If any precipitation can be seen at this point, break the precipitation by pipetting up and down 10 times.

11. Assemble a HiBind® DNA Mini column in a 2 ml collection tube (provided). **Transfer the entire sample from Step 10 into the column**, including any precipitate that may have formed. Centrifuge at 10,000 x g for 1 min to bind DNA. Discard the collection tube and filtrate.

12. **Place the column into a second 2 ml tube and wash by adding 500 µl Buffer HB.** Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse the collection tube.

13. **Place the column into the same collection tube and wash by adding 700 µl DNA Wash Buffer diluted with ethanol**. Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse the collection tube.

    NOTE: DNA Wash Buffer is supplied as a concentrate and must be diluted with absolute ethanol according to the instructions on Page 4, under "Before Starting."

14. **Wash the column with a second 700 µl DNA Wash Buffer and centrifuge as above**. Discard flow-through and reuse the collection tube.

15. **Using the same 2 ml collection tube, centrifuge HiBind® DNA Mini Column at maxi speed (≥10,000 x g) for 2 min to dry the column**. *This step is critical for removal of trace ethanol that might otherwise interfere with down stream applications.*

16. **Place the column into a nuclease-free 1.5 ml microfuge tube and add 50-100 µl of preheated (65°C) Elution Buffer to HiBind® DNA Mini column matrix**. Allow columns to incubate for 3 to 5 min at room temperature after addition of Elution Buffer.
    NOTE: Incubating the HiBind® DNA column at 65°C rather than at room

temperature prior to centrifugation will give a modest increase in DNA yield per elution.

17. **To elute DNA from the column, centrifuge at 10,000 x g for 1 min.**

18. Repeat the elution with a second 50-100 ul Elution Buffer.

Note: Each 50-100µl elution typically yields 60-70% of the DNA bound to the column. Thus two elutions generally give ~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. In some instances yields may be increased by incubating the column at 70°C (rather than at room temperature) upon addition of Elution Buffer.

The expected yield from a 3 ml culture sample is 15-30 µg DNA depending on bacterial strain, medium, and growth phase.

E.Z.N.A.™ Bacterial DNA Vacuum/Spin Protocol

Note: Please read through previous section of this manual before using this protocol.

1. Prepare samples by following the standard Protocol in previous section (Steps 1-10).
2. Prepare the vacuum manifold according to manufacturer's instructions and connect the V-Spin column to the manifold.
3. Load the sample/BDL/Ethanol mixture to the column. Switch on vacuum source to draw the sample through the column and turn off the vacuum.
4. Wash the column by adding 500 µl Buffer HB, draw the buffer HB through the column by turning on the vacuum source.
5. Wash the column by adding 700 µl DNA Wash Buffer, draw the wash buffer through the column by turning on the vacuum source. Repeat this step with another 650 µl DNA wash buffer.
6. Proceed step 15-18 of E.Z.N.A.™ Bacterial DNA Spin Protocol on page 6-7.

Troubleshooting Guide

Problem	Possible Cause	Suggestions
Clogged column	Incomplete lysis	Add the correct volume of Buffer BTL and incubate at 55°C to obtain complete lysis. It may be necessary to extend incubation time to 30 min.
	Sample too large	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 min.
Low DNA yield	Clogged column	See above
	Poor elution	Repeat elution or increase elution volume (see note on page 6). Incubation of column at 65°C for 5 min after addition of Elution Buffer may increase yields.
	Improper washing	DNA Wash Buffer Concentrate must be diluted with absolute (96%-100%) ethanol
Low A_{260}/A_{280} ratio	Extended centrifugation during elution step.	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation — it will not interfere with PCR or restriction digests.
	incomplete mixing with Buffer BDL	Repeat the procedure, this time making sure to vortex the sample with Buffer BDL immediately and completely.
	insufficient incubation.	Increase incubation time with Buffer BTL. Ensure that no visible cell clumps remain.
	Trace protein contaminants remain.	Following step 12, wash column with 300 µl Buffer HB before proceeding to step 13.
No DNA eluted	Poor cell lysis due to improper mixing with Buffer YDL.	Mix thoroughly with Buffer BDL and incubate at 70°C prior to adding ethanol.
	Incomplete spheroblasting	Add more lysozyme or extend the incubation time. It may be necessary to increase incubation by 15 min.
	Absolute ethanol not added to lysate/Buffer BDL mixture.	Before applying sample to column, an aliquot of ethanol must be added. See protocol above.
	No ethanol added to Wash Buffer.	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.