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## Introduction

E.Z.N.A.™ Bacterial DNA Kits allow rapid and reliable isolation of high-quality total cellular DNA from a wide variety of Bacterial species. Up to  $1 \times 10^9$  Bacterial cell can be processed. The system combines the reversible nucleic acid-binding properties of HiBind™ matrix with the speed and versatility of spin column technology to yield 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: This E.Z.N.A.™ Bacterial DNA Kits 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 procedure. Bacterial cells are grown to log-phase and harvested. Bacterial cell wall is removed by lysozyme digestion and followed by protease K digestion. Following lysis, binding conditions are adjusted and the sample applied to a HiBind® DNA spin-column. Two rapid wash steps remove trace salt 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.

## New in this edition

- Newly introduced V-Spin column (#D3485) features an attached cap and a standard outlet luer at the bottom. The attached cap virtually assures the elimination of potential contamination.
- Optional vacuum/spin protocol is available for V-Spin column

## Storage and Stability

All components of the E.Z.N.A.™ Bacterial DNA Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer BDL. It is possible to dissolve such deposits by warming the solution at 37°C, though they do not interfere with overall performance.

## Kit Contents

Product	D3450-00 D3350-00 Trial Sample	D3450-01 D3350-01 50 Preps	D3450-02 D3350-02 200 Preps
<b>Components</b>			
HiBind™ DNA columns	5	50	200
2 ml Collection Tubes	10	100	400
Buffer BTL	1.5 ml	20 ml	50 ml
Buffer BDL	5 ml	25 ml	100 ml
Wash Buffer Concentrate	12 ml	40 ml	2 x 60 ml
Lysozyme	5 mg	50mg	4 x 50mg
Protease K	2.0 mg	19 mg	78 mg
RNase A	55µl	530µl	4.20ml
User Manual	1	1	1



Buffer BDL contains a chaotropic salt. Use gloves and protective eyewear when handling this solution.

## Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.™ Bacterial DNA procedure.
- Prepare a stock solution of Proteinase K (provided) as following and aliquot into adequate portions. Store aliquots at -20°C.
  - D3450-00, D3350-00 dissolve with 130 µl of TE Buffer
  - D3450-01, D3350-01 dissolve with 1.30 ml of TE Buffer
  - D3450-02, D3350-02 dissolve with 5.1 ml of TE Buffer
- Prepare an 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.
  - D3450-00, D3350-00 dissolve with 100 µl of TE Buffer
  - D3450-01, D3350-01 dissolve with 1 ml of TE Buffer
  - D3450-02, D3350-02 dissolve with 1 ml of TE Buffer

- Dilute DNA Wash Buffer Concentrate with ethanol as follows and **store at room temperature.**

D3450-00, D3350-00 Add 18 ml absolute (96%-100%) ethanol

D3450-01, D3350-01 Add 60 ml absolute (96%-100%) ethanol

D3450-02, D3350-02 Add 90 ml absolute (96%-100%) ethanol/bottle

## Bacterial DNA Miniprep Protocol

Have the following reagents and supplies ready:

- Tabletop microcentrifuge and nuclease-free 1.5 ml tubes.
  - waterbath set to 30°C.
  - Shaking waterbath set to 55°C.
  - Sterile dH<sub>2</sub>O (~0.5 ml per sample) equilibrated to 70°C.
  - Absolute ethanol - do not use other alcohols.
  - Molecular Biology grade Lysozyme (10mg/ml in TE buffer).
- 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 case.)
  - Harvest no more than 3 ml culture by centrifugation at 4,000 x g for 10 min at room temperature.
  - Discard medium and resuspend cells in 180 µl TE buffer. **Add 20 µl of 50mg/ml lysozyme followed by 10 min incubation at 30°C.**  
**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
  - Pellet digested cell by centrifuging 5 min at 5,000 x g at room temperature.
  - Resuspend cell in **200 µl Buffer BTL. Add 25 µl of a proteinase K solution.** Vortex to mix well, and incubate at 55°C in a shaking water bath

to effect complete lysis. Usually no more than 1 h is required for bacteria lysis. If no shaking waterbath is available, vortex the sample every 20-30 minutes.

6. Add 20 µl RNase A (20 mg/ml) and incubate at room temperature for 2 minutes.
7. Add 220 µl Buffer BDL and vortex to mix. Incubate at 70°C for 10 minutes. A wispy precipitate may form upon addition of Buffer BDL, but it does not interfere with DNA recovery.
8. Add 220 µl absolute ethanol and mix thoroughly by vortexing.
9. Assemble a HiBind® DNA spin-column in a 2 ml collection tube (provided). Transfer the entire sample from step 8 into the column including any precipitate that may have formed. Centrifuge at 8,000 x g for 1 min to bind DNA. Discard the collection tube and filtrate.
10. Place the column into a **second 2 ml tube** and wash by pipetting 650 µl of DNA Wash Buffer diluted with ethanol. Centrifuge at 8,000 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 3, under "**Before Starting.**"
11. Wash the column with a second 650 µl of Wash Buffer and centrifuge as above. Discard flow-through and reuse the collection tube.
12. Using the same empty 2ml collection tube, centrifuge the spin-column at maximum speed ( $\geq 10,000$  x g) for 2 min to dry the column. *This step is crucial for ensuring optimal elution in the following step.*
13. Place the column into a sterile 1.5 ml microfuge tube and add 100µl of preheated (70°C) sterile dH<sub>2</sub>O or 10 mM Tris buffer, pH 8.5. Allow tubes to sit for 1 min at room temperature.
14. To elute DNA from the column, centrifuge at 8,000 x g for 1 min. Repeat the

elution with a second 100 µl of pre-heated dH<sub>2</sub>O if necessary.

**NOTE:** Incubation of the HiBind® DNA column loaded with 200 µl dH<sub>2</sub>O or Tris buffer at 70°C rather than at room temperature, prior to centrifugation will give a modest increase in DNA yield per elution. Yields may further be increased by eluting a third time with the same 200 µl containing DNA.

### Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water or Tris-HCl buffer as blank. DNA concentration is calculated as:

$$[DNA] = (Absorbance_{260}) \times (0.05 \mu\text{g}/\mu\text{l}) \times (\text{Dilution factor})$$

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A ratio of ( $A_{260}/A_{280}$ ) of 1.7-1.9 corresponds to 85%-95% purity.

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

If DNA is eluted with dH<sub>2</sub>O rather than Tris buffer, store the sample at -20°C to prevent degradation.

### Vacuum/Spin Protocol for Bacterial DNA Isolation (V-Spin column only)(#D3350)

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

1. **Prepare samples by following the standard Protocol in previous sections (step 1-8).**
2. Prepare the vacuum manifold according to manufacturer's instruction and connect the V-Spin column to the manifold.
3. Load the sample/BDL/Ethanol mixture to the column.
4. Switch on vacuum source to draw the sample through the column and turn off the vacuum.
5. Wash the column by adding 750 µl DNA wash buffer, draw the wash buffer through the column by turn on the vacuum source. Repeat this step with another 750 µl DNA wash buffer.
6. **Assemble the column into a 2 ml collection tube and transfer the column to a micro centrifuge. Spin 1 minute to dry the column.**
7. Place the column in a clean 1.5 ml microcentrifuge tube and add 30-50µl TE or water. Stand for 1-2 minute and centrifuge 1 minute to elute DNA.

## Troubleshooting

Problem	Possible Cause	Suggestions
Clogged Column	Incomplete lysis	Add the correct volume of Buffer BTL and incubate at 70°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 <sub>600</sub> 10 or 1 x 10 <sup>9</sup> 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 70°C for 5 min with dH <sub>2</sub> O or Tris buffer may increase yields.
	Improper washing	DNA Wash Buffer Concentrate must be diluted with absolute (100%) ethanol as specified on page 3 before use.
Low A <sub>260</sub> /A <sub>280</sub> 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.
	Poor cell lysis due to incomplete mixing with Buffer BDL	Repeat the procedure, this time making sure to vortex the sample with Buffer BDL immediately and completely.
	Incomplete cell lysis or protein degradation due to insufficient incubation.	Increase incubation time with Buffer BTL. Ensure that no visible cell clumps remain.
	Trace protein contaminants remain.	Following step 8, wash column with 300 µl Buffer BDL before proceeding to step 9.

Problem	Possible Cause	Suggestions
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 Concentrate.	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.

## Other Product Information

Product No.	Product Name	Description
Plant DNA and Plant RNA Isolation Kits		
D3485-01/02 D3486-01/02	Plant DNA Miniprep Kit	Isolation of total cellular DNA from dry and wet plant samples
R6627-01/02 R6827-01/02	Plant RNA Kit	Isolate total cellular RNA from plant samples
Fungal DNA and Fungal RNA Kit		
D3490-01/02 D3390-01/02	Fungal DNA Miniprep Kit	Isolation of total cellular DNA from dry and wet fungal samples
D3590-01/02	Fungal DNA Midiprep Kit	Isolation of total cellular DNA from up to 500 mg fungal samples
R6640-01/02 R6840-01/02	Fungal RNA Kit	Isolate total cellular RNA from fungal samples
Yeast, Bacterial, Mollusc DNA Kits		
D3473-01/02	Mollusc DNA Kit	Isolate genomic DNA from mollusc, arthropods, worms
D3450-01/02	Bacterial DNA Kit	Isolation genomic DNA from bacterial.
D3476-01/02	Yeast Plasmid Kit	Isolate yeast plasmid.