

E.Z.N.A.[®] Bacterial DNA Kit

– Instruction Manual –

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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 1×10^9 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 A260/A280 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.

Theory

Bacterial cells are grown to logphase 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.

* PCR is covered by patents owned by F. Hoffmann-La Roche Ltd.

Kit Components

E.Z.N.A.® Bacterial DNA Mini Kit	5 Purifications	50 Purifications	200 Purifications
Product Number	12-3450-00	12-3450-01	12-3450-02
Components			
HiBind® 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
DNA Wash Buffer	12 ml	40 ml	3 x 40 ml
Lysozyme	5 mg	50 mg	200 mg
OB™-Protease	2 mg	19 mg	76 mg
RNase A	55 µl	530 µl	2.1 ml
10 mM TE Buffer	1.5 ml	10 ml	30 ml
Elution buffer	1.5 ml	15 ml	60 ml

Storage and Stability

RNase A should be stored at 4°C, aliquots of Lysozyme at -20°C.

All the other components of E.Z.N.A.® Bacterial DNA Mini Kit components should be stored at room temperature (22 °C – 25 °C). All E.Z.N.A.® Bacterial DNA Mini Kit components are stable for at least 12 months from the date of purchase when stored at 22-25 °C.

Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

- ! Buffer BDL contains a chaotropic salt. Use gloves and protective eyewear when handling this solution.
- ! Prepare OB-Protease stock solution as following: 2 mg dissolve with 130µl of TE Buffer (provided); 19 mg dissolve with 1.3 ml of TE Buffer (provided); 78 mg dissolve with 5.2 ml of TE Buffer (provided) - and aliquot into adequate portions. Store each aliquot at -20°C and thaw before use.
- ! Prepare Lysozym stock solution as following: 5 mg dissolve with 500µl of TE Buffer; 50 mg dissolve with 5 ml of TE Buffer (not provided); 200 mg dissolve with 20 ml of TE Buffer - and aliquot into adequate portions. Store each aliquot at -20°C and thaw before use.
- ! DNA Wash Buffer is concentrated and has to be diluted with absolute ethanol as follows:

Kit 12-3450-00	Add 18 ml 100% EtOH to 12 ml Wash Buffer
Kit 12-3450-01	Add 60 ml 100% EtOH to 40 ml Wash Buffer
Kit 12-3450-02	Add 3 x 60 ml 100% EtOH to 3 x 40 ml Wash Buffer

Store diluted DNA Wash Buffer at room temperature.

E.Z.N.A.[®] Bacterial DNA Isolation Protocol

Materials required, but not supplied:

- ! Tabletop microcentrifuge and nuclease-free 1.5 ml tubes.
- ! waterbath set to 30°C.
- ! Shaking waterbath set to 55°C.
- ! Sterile dH₂O (~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).

1. Homogenization and lysis

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.)

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 100 µl TE buffer. Add 100 µl of 10mg/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 OB-Protease 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.

2. RNase Digest

Add 10 µl RNase A and incubate at room temperature for 2 minutes.

3. Load and Bind

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.

Add 220 µl absolute ethanol and mix thoroughly by vortexing.

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.

4. Wash I

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.

5. Wash II

Repeat the washing step as described in step 3 with 650 µl of the completed DNA Wash Buffer. Discard the flow-through liquid and keep the spin column for the next step.

6. Dry (Important, do not skip this step!)

Place the HiBind® spin column containing your DNA in the collection tube used in step 4 and centrifuge for 2 min at maximum speed to dry the column matrix .

7. Elution

Place the column into a sterile 1.5 ml microfuge tube and add 100µl of preheated (70°C) Elution buffer or sterile dH₂O. Allow tubes to sit for 1 min at room temperature. 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 dH₂O or TE buffer if necessary.

Quantitation and storage of DNA

Determine the absorption of an appropriate dilution (10- to 50-fold) of the sample at 260 nm and then at 280 nm.

One A_{260} -unit is about 50 μg DNA/ml. The DNA concentration is calculated as follows:

$$\text{DNA conc. } (\mu\text{g / ml}) = \text{Absorption}_{260} \times 50 \times \text{Dilution Factor}$$

The ratio of $A_{260/280}$ is an indication of nucleic acid purity. A value higher than 1.8 indicates > 90% nucleic acid.

Phenol has an absorption maximum at 275 nm and can interfere with absorption readings of DNA or RNA. However, the E.Z.N.A.[®] Plant DNA Kit eliminates the use of phenol and avoids this problem.

Store DNA samples at $-20\text{ }^{\circ}\text{C}$ in 10 mM Tris-HCl (pH 8.5) or sterile dH_2O . Under such conditions DNA prepared with the E.Z.N.A.[®] system is stable for years.

Ordering information

For DNA isolation from bacterial

E.Z.N.A. [®] Bacterial DNA Mini Kit	12-3450-00	5 Preparations
	12-3450-01	50 Preparations
	12-3450-02	200 Preparations
E.Z.N.A. [®] Bacterial RNA Mini Kit	12-6850-00	5 Preparations
	12-6850-01	50 Preparations
	12-6850-02	200 Preparations

Troubleshooting Tips

Problem	Likely cause	Suggestion
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 OD600 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 70°C for 5 min with dH ₂ 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 A260/A280 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.

