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BIO BASIC INC.

EZ-500 Spin Column Bacteria Genomic DNA Maxi-Preps Kit

PT92038

Version 4.0
ISO9001 Certified

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Product information for PT92038:

Kit Contents

Components	PT92038, 4 Preps
Universal Digestion Buffer	40 ml
Universal Buffer BD	50 ml
Universal PW Solution	18 ml
Universal Wash Solution	7.5 ml
CE Buffer (pH 9.0)	15 ml
Proteinase K (10mg/ml)	5 ml
EZ-500 Column	4
50 ml Collection Tube	4
Protocol	1

Note 1: Universal Buffer BD contains chaotropic salt. Avoid contact with skin and eyes.

Note 2: Universal PW Solution and Universal Wash Solution are supplied as concentrates. Add **12 ml isopropanol** to **18 ml Universal PW Solution** and **30 ml ethanol (96-100%) for 7.5 ml Universal Wash Solution** before use to obtain a working solution.

Storage and Stability

EZ-500 columns and all buffers should be stored dry, at room temperature (15-25 °C) and are stable for 1 year under these conditions. Proteinase K is supplied as 10 mg/ml solution, the solution can be keep at Room Temperature for 6 months, for long-term storage keep at -20 °C.

Introduction

The kit provides a simple and convenient technique for large scale isolation of high quality DNA from both Gram negative and Gram positive bacteria. Up to 500 ug of DNA of cell lysate is selectively bound to EZ-500 spin column. Other impurities such as proteins, salts do not bind on the column and are eliminated in flow through. No phenol extraction, no ethanol precipitations are required. The kit is also suitable for isolation of bacterial genomic DNA from colonies on dish. Purified DNA is suitable for downstream applications such as Restriction Endonuclease Digestions, PCR, and other applications.

Features

1. Suitable for both Gram positive and Gram negative bacteria.
2. Fast. Using a rapid spin-column format, the entire procedure takes 30 minutes.
3. High quality of DNA. Purified DNA has an OD_{260}/OD_{280} ratio between 1.7 and 1.9.

Reagents and Materials Supplied by User:

Microcentrifuge capable of at least 12,000 × *g*
Pipets and pipet tips
Vortexer
Isopropanol
Ethanol (96-100%)
Lysozyme (for Gram positive bacterial)
RNase A (20 mg/ml, Optional for RNA-free DNA)
Microcentrifuge tubes (50 ml)
Water bath for heating at 56 °C

Important Notes Before Starting

This protocol is designed for purification of total DNA from Gram positive or Gram negative bacteria. All centrifugation steps are carried out at room temperature (15-25°C) in a microcentrifuge. It is strongly advised that this protocol should be read thoroughly before starting.

- **ü** Proteinase K is supplied in a ready-to-use solution form, but RNase A is not provided in this kit, if RNA-free DNA is required, please prepare RNase solution and see protocol to add the RNA removal step.
- **ü** For Gram Positive bacteria, cell wall should be removed by an enzyme (e.g. Lysozyme) before lysis, but the enzyme is NOT supplied in the kit.
- **ü** Check the Universal Buffer Digestion and Universal Buffer BD for salt precipitation before each use. If necessary, redissolve the precipitate by warming the solution at 56 °C, then cool back down to room temperature before use.

- ü CE Buffer is 10 mM Tris-HCl, 0.5 mM EDTA, pH 9.0. Water can be used as eluate in the final step if EDTA should be avoided for the following applications, but it is not recommended if the pH of water is less than 7.0.
- ü Preheat the water bath or rocking platform to 56 °C.

Procedures

1. Sample Preparation.

A. **Gram-negative bacteria** (*E. coli*, *streptococcus*, *pneumococcus*, *etc.*)

Transfer 30-50 ml overnight culture into 50 ml centrifuge tube and centrifuge at 10,000 *x g* for 3 minutes, discard supernatant. Proceed to Step 2.

B. **Gram-positive bacteria** (*staphylococcus*, *Corynebacterium diphtheriae, etc.*)

a. Transfer 30-50 ml overnight culture into 50 ml centrifuge tube and centrifuge at 10,000 *x g* for 3 minutes, discard supernatant.

b. Add 9 ml lysozyme solution (20 mg/ml lysozyme, 20 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 1% Triton X-100. NOT supplied in the kit), suspend thoroughly and incubate at 37 °C for 30-60 minutes. Centrifuge at 10,000 *x g* for 3 minute, discard the supernatant. Proceed to Step 2.

2. Add 9 ml Buffer Digestion and 1 ml Proteinase K to the sample. Vortex and mix thoroughly. Incubate at 56°C for 1 h.

Note: If RNA-free genomic DNA is required, add 1 ml RNase A (20 mg/ml), mix by vortexing, and incubate for 2 min at room temperature before continuing with Step 3.

3. Add 10 ml Buffer BD, mix thoroughly by vortexing.

Note: If a gelatinous material appears at this step, incubate at 70 °C for 10 min.

4. Add 10 ml ethanol (96-100%), mix thoroughly by vortexing.

Note: If a gelatinous material appears at this step, vigorously shaking or vortexing is recommended.

5. Transfer the mixture from step 4 (including any precipitate) into the EZ-500 spin column placed in a collection tube. Centrifuge at 9,000 *x g* (12,000 *rpm*) for 1 minute. Discard the flow-through.

6. Add 5 ml Universal PW Solution, and centrifuge for 1 min at 9,000 x *g* (12,000 *rpm*). Discard the flow-through.

Note: Check the label to ensure Universal PW Solution was diluted with isopropanol.

7. Add 5 ml Universal Wash Solution, and centrifuge for 1 min at 9,000 x *g* (12,000 *rpm*). Discard the flow-through.

Note: Check the label to ensure Universal Wash Solution was diluted with ethanol.

8. Centrifuge for an additional 2 minutes at 9,000 *x g* (12,000 *rpm*) to dry the EZ-500 membrane. Discard flow-through and transfer the spin column to a clean 1.5 ml centrifuge tube.

Note: It is important to dry the membrane of the EZ-500 spin column, since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during the

following elution.

9. Add 1 ml Buffer CE directly onto the center part of EZ-500 membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at 9,000 x *g* (12,000 *rpm*) to elute the DNA.

Note 1: Warm the Buffer CE to 60 °C will increase the elution efficiency.

Note 2: For maximum DNA yield, repeat elution once as described in this step.

Note 3: A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first elute.