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PRODUCT INFORMATION

Rapid Bacteria Genomic DNA Isolation Kit

Product information for BS8225:

Kit Contents

Components	BS8225, 50 Preps
Universal Digestion Buffer	25 ml
Buffer PB	12 ml
TE Buffer	10 ml
Protocol	1

Storage and Stability

Store kit at room temperature. Valid for 1 year.

Introduction

The kit is designed for rapid small-scale extraction of high quality genomic DNA from a variety of Gram-negative or Gram-positive bacteria. Purified DNA can be used for many downstream applications such as PCR, restriction enzyme digestion, hybridization and other applications.

Features

1. Rapid and Simple.
2. High Quality of DNA. OD_{260}/OD_{280} of purified DNA is generally

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1.8~1.9.
3. No Toxic Substance. The kit does not contain toxic reagents.
4. Easy to Scale Up.

Procedures

1. Sample Preparation.

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

a. Transfer 1 ml overnight culture (about 2 x 10^9 cells) into a centrifuge tube and centrifuge at 10,000 x *g* for 30 seconds, discard supernatant.

b. Add 400 µl Universal Buffer Digestion into the pellets, vortex and incubate at 65 °C until cells are lysed thoroughly.

Note 1: Usually incubation is 30~60 minutes. If RNA-free DNA is needed, add 20 µl RNase A (20 mg/ml. NOT supplied in the kit) and incubate at room temperature for 5 minutes before step 3.

Note 2: Buffer Digestion may form precipitates during long-term storage. Warm the bottle at 65 °C to dissolve the precipitates

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

a. Transfer 1 ml overnight culture (about 2 x 10^9 cells) into a centrifuge tube and centrifuge at 10,000 x g for 30 seconds, discard supernatant.

b. Add 180 µl lysozyme solutions (20 mg/ml lysozyme, 20 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 1% Triton X-100. NOT supplied in the kit). Mix thoroughly and incubate at 37 °C for 30-60 minutes. Centrifuge at 10,000 x *g* for 1 minute, discard the supernatant.

c. Add 400 µl Universal Buffer Digestion into the pellets,

vortex and incubate at 65 °C until cells are lysed thoroughly.

Note 1: Usually incubation is 30~60 minutes. If RNA-free DNA is needed, add 20 µl RNase A (20 mg/ml. NOT supplied in the kit) and incubate at room temperature for 5 minutes before step 3.

Note 2: Buffer Digestion may form precipitates during long-term storage, warm the bottle at 65 °C to dissolve the pellet.

3. Add 200 µl Buffer PB, mix by inverting. Incubate at -20 °C for 5 minutes.

4. Centrifuge at 12,000 x *g* for 5 minutes at room temperature. Transfer the supernatant to a new 1.5 ml tube.

5. (Optional) Add 0.2 ml of chloroform to the supernatant, mix well by inverting 10 times. Centrifuge at 12,000 x *g* for 2 minutes. Carefully transfer the supernatant to a clean 1.5 ml tube.

6. Add equal volume of isopropanol (approx 0.3~0.5 ml) to the solution, mix well by inverting 5 times. Incubate at room temperature for 2~5 minutes. Centrifuge at 12,000 x *g* for 5 minutes, discard the supernatant carefully.

7. Add 1 ml of pre-cooled 75% ethanol to the pellet, mix well by inverting 10 times. Centrifuge at 12,000 x *g* for 1 minute, discard the supernatant.

8. Repeat Step 7.

9. Air-dry the pellet at room temperature with the lid open for 2~5 minutes.

Note: Don't over dry.

10. Add 50~200 µl of TE (10 mmol/L Tris, 1 mmol/L EDTA, pH 8.0) buffer to dissolve DNA pellet. Keep at 4 °C for a couple hours until DNA pellet is completely dissolved. The purified DNA is ready for use. Or keep at -20 °C for long term storage.