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
Transportation at ambient temperature. Upon receipt, store kit at 4°C. 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.

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2. High Quality of DNA. OD_{260}/OD_{280} of purified DNA is generally 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.

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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.

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