MQ Bacterial Genomic DNA Extraction Kit

Cat No. BDE-M-002-50

Size: 50 Preparations

MOLEQULE-ON®

Component	Size
Solution A	15 ml
Solution B	15 ml
Wash Solution*	15 ml
Elution Buffer	10 ml
Proteinase K	1 ml
RNase A	1 ml
MQ Columns and 2ml Collection Tubes	50 Each

Preparations

* NOTE: Please add 60ml of Absolute Ethanol to the 15ml of Wash Buffer before use.

Storage

MQ Bacterial DNA Extraction Kit should be store in dry condition (15℃-25℃), re-test period for 12 months. Storage at 2-8℃ for longer. RNase A and Proteinase K should be store at -20℃.

Description

The kit provides a simple and convenient technique to isolate high quality DNA from both Gram negative and Gram positive bacteria using a rapid spin-column format. DNA of cell lysate is selectively bound to the spin column and 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 genomic DNA can be up to 50 kb in length. Purified DNA is suitable for downstream applications such as Restriction Endonuclease Digestions, PCR, and other applications.

Features

- Fast and easy processing using a rapid spin-column format. The entire procedure takes approximately 30 minutes.
- High yield. 5-20 µg of bacterial genomic DNA can be obtained from 1 ml (10^8-10^9 cells) of overnight culture.
- High quality of DNA. $OD_{260}/_{280}$ of purified DNA is generally 1.7-1.9.
- No phenol/chloroform extraction or ethanol precipitation is required.

Materials Supplied by User

Microcentrifuge capable of at least 12,000 × g, Pipettes and pipette tips, Vortexer, Ethanol (96-100%), Lysozyme (for Gram positive bacteria), Microcentrifuge tubes (1.5 ml or 2 ml), Water bath for heating at 56°C

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 you read this protocol thoroughly before starting. MQ Column Bacteria DNA Extraction Kit is designed to be simple, fast and reliable provided that all steps are followed diligently. Prepare all components, and have the necessary materials as outlined before starting.

Procedure

1. Sample Preparation

A. Gram-negative bacteria (E. coli, streptococcal, pneumococcal, etc.).
Transfer overnight culture (about 2 x 10^9 cells) into centrifuge tube and centrifuge at 12000 x g for 60 seconds, discard supernatant.
B. Gram-positive bacteria (golden staphylococcal, orynebacteriadiphtheriae, etc.).
Transfer overnight culture (about 2 x 10^9 cells) into centrifuge tube and centrifuge at 12000 x g for 60 seconds, discard supernatant.

1. Completely resuspend the bacterial pellet in 200µl of Solution A, add 20µl RNase A, mix thoroughly and incubate at RT for 15-30min.

Note: Add 200 µl lysozyme solution (20 mg/ml lysozyme, 20 mM Tris-HCl, pH 8.0, 2mM EDTA, 1.2% Triton X-100. NOT supplied in the kit) for Gram-positive bacteria, suspend thoroughly and incubate at 37°C for 30-60 minutes.

2. Add 20 µl Proteinase K (10mg/ml) and mix thoroughly by vortexing. Incubate at 56°C for 30-60 min. Invert the tube several times during incubating. The mixture should be clear.
3. Add 200µl Solution B, Mix thoroughly. White precipitates may form, incubate at 75℃ for 15-30 min, White precipitates will disappear. It shows that the sample does not digested thoroughly if the solution not clear, which may make the amount and purity of DNA extraction lower and block the column.
4. Add 200µl ethanol (96-100%) to the sample, and mix thoroughly by vortex. A white precipitate may form on addition of ethanol. Add the mixture into a MQ Adsorption column, incubate at RT for 2 min.
5. Centrifuge for 2 min at 12,000 rpm. Discard the flow-through and re-use the collection tube in the next step.
6. Wash the MQ Adsorption Column with 600µl Washing buffer, centrifuge at 12,000 rpm for 1 min, discard the flow-through and re-use the collection tube in the next step.

Note: Washing buffer must be diluted with absolute ethanol before use.

7. Repeat step 6 with another 600µl Washing buffer.
8. Centrifuge the empty column at 12,000 rpm for 2 min. Incubate at room temperature or 50℃ to dry the column. It is critical for removing ethanol from the column.
9. Place column into a new clean centrifuge tube. Add 50-200µl Elution buffer (been heated to 65℃) onto the column matrix, incubate at room temperature for 5 min. Centrifuge at 12,000 rpm for 1 min.
11. For increased DNA concentration, add the solution obtained from step 10 to the center of membrane again. Incubate at room temperature (15-25℃) for 2-5 min, and then centrifuge for 2 min at 12,000 rpm.

Troubleshooting

1. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size.
2. If the precipitation appears, dissolve at 65℃ water bath please, which had not affect on using.
3. If Adsorption Column is blocked , the time of centrifugation can be extended.
4. If the volume of eluted buffer is less than 50µL, it may affect recovery efficiency. The pH value of elution buffer will have big influence in eluting. If using distilled water, pH should be controlled at 8.0, below 7.0 will affect elution efficiency. DNA product should be stored at -20°C to avoid degradation.
5. Detect the concentration and purity of DNA: The purity of plasmid DNA influenced by many factors, the DNA purity can be detected by Agarose gel electrophoresis and Ultraviolet spectrophotometer. DNA should have absorption peak in OD_{260}, OD_{260}=1 is equal to 50µl double-stranded DNA, 40µl single-stranded DNA. OD_{260}/OD_{280} should be 1.7-1.9, the value will be lower if using distilled water in eluting, but do not show the purity is low.