MQ Bacterial DNA extraction Kit

Cat No. BDE-M-001-50 (50 Preps)
BDE-M-001-250 (250 Preps)

MOLEQULE-ON®

Component	BDE-M-001-50 (50 Preps)	BDE-M-001-250 (250 Preps)
Universal Buffer Digestion	10 ml	50 ml
Universal Buffer BD	12 ml	60 ml
Universal PW Solution (concentrate)	18 ml	90 ml
Universal Wash Solution (concentrate)	7.5 ml	37.5 ml
CE Buffer	15ml	75 ml
Proteinase K	1.2ml	6 ml
MQ Columns and 2ml Collection Tubes	50 Each	250 Each

Preparations

NOTE 1: Universal Buffer BD contains a 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 22.5 ml ethanol (96-100%) for 7.5ml Universal Wash Solution before use to obtain a working solution.

Storage

MQ Bacterial DNA Extraction Kit should be stored dry at 15°C - 25°C. The proteinase K should be stored at – 20°C.

Description

MQ Bacterial DNA Extraction 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, Isopropanol, Ethanol (96-100%), Lysozyme (for Gram positive bacteria), Rnase A (20 mg/ml, Optional for RNA-free DNA), 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 Genomic DNA Purification 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.

Proteinase K is supplied in a ready-to-use solution form, but RNase A is not provided in this kit, if RNA-free DNA are required, please prepare RNA solution and see protocol to add the RNA removal step. 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.
Check the Universal Buffer Digestion and Universal Buffer BD for salt precipitation before each use. If necessary, re-dissolve the precipitate by warming the solution at 56°C, then cool back down to room temperature before use.
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.
Universal PW Solution and Universal Wash Solution are supplied as concentrates. Before using for the first time, add 12 ml isopropanol to 18 ml Universal PW Solution, 22.5 ml ethanol to 7.5 ml Universal Wash Solution, respectively.
Preheat the water bath or rocking platform to 56°C.

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 10000 x g for 30 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 10000 x g for 30 seconds, discard supernatant.

- Add 180 µl 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. Add 20 µl Proteinase K and mix thoroughly by vortexing. Incubate at 56°C for 30 min. Continuing with step 3.

2. Add 180 µl Universal Buffer Digestion and 20 µl Proteinase K to the sample, and mix thoroughly by vortexing. Incubate at 56°C for 1 hour.
NOTE: If RNA-free genomic DNA is required, add 20 µl RNase A (20 mg/ml), mix by vortexing, and incubate for 2 minutes at room temperature then continue with step 3.
3. Add 200 Universal Buffer BD, mix thoroughly by vortexing. Incubate at 70°C for 10 minutes.
4. Add 200 µl 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 MQ column placed in a 2 ml collection tube. Centrifuge at 9,000 x g (12,000 rpm) for 1 minute. Discard the flow-through.
6. Add 500 µl Universal PW Solution, and centrifuge for 1 minute 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 500 µl Universal Wash Solution, and centrifuge for 1 minute 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. Place the empty column in the microcentrifuge and centrifuge for an additional 2 minutes at 9,000 x g (12,000 rpm) to dry the MQ 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 MQ 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 50-100 µl Buffer CE directly onto the center part of MQ membrane. Incubate at room temperature for 1 minute, and then centrifuge for 1 minute 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: Elution with more than 100 µl (e.g. 200 µl) increases the DNA yield, but the concentration will be lower.
NOTE 3: For maximum DNA yield, repeat elution once as described in this step.
NOTE 4: A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate.
NOTE 5: For maximum DNA concentration, use the eluate in the microcentrifuge tube for the second elution step.