MQ Bacterial DNA Isolation Kit

Cat No. BDE-M-001-50
Size: 50 Preparations

MOLEQULE-ON®

Kit Components	Volume
Digestion Solution	20 ml
Wash Solution (A)	12 ml
Elution Buffer	5 ml
Proteinase K (B)	2 mg
MQ-50 Column with 2ml collection tubes	50

Preparations

(A) Before use, add 48 ml of 100% ethanol to 12 ml **Wash Solution**.
(B) Before use, add 150µl of sterilized water to the tube containing 2mg of **proteinase K**.
(C) **Digestion Solution** may form a precipitate upon storage. Dissolve the precipitate by warming the solution to 37°C if necessary.
(D) **Elution Buffer** is 2 mM Tris-HCl pH 8.0.

Storage

MQ Bacterial Isolation Kit should be stored dry at 15°C - 25°C. The proteinase K included in the kit should be stored at – 20 °C.

Description

MQ Bacterial DNA Isolation Kit with Spin Columns provides a fast, simple and efficient method for purification of genomic DNA from various bacteria. MQ kits take advantage of silica based DNA purification technology, where DNA is selectively adsorbed in the silica-based membrane embedded in MQ Spin Column. Other components and impurities flow through the column. Genomic DNA is then eluted off the column and can be readily used in most downstream applications. Hazardous compounds such as phenol, chloroform, or CsCl are not required.

Features

- MQ Bacterial Isolation Kit procedure is simple, fast and efficient.
- It prepared high quality DNA that can be used in any downstream applications such as sequencing, PCR, cloning, transformation or restriction digestions.
- The protocol is reproducible.
- It gives high yield of DNA, Up to 10µg of DNA per column.

Principle

MQ Bacterial Isolation Kit utilizes a silica- gel membrane that selectively absorbs up to 10 µg of DNA fragments in the presence of specialized binding buffers. Nucleotides, oligos (less than 40 bases), enzymes, mineral oil and other impurities do not bind to the membrane and are washed away. The DNA fragments can then be eluted off the column in small volume and used in downstream applications without further processing.

Procedure

1. Spin appropriate number of bacteria (about 10^6 ~10^7) at 6,000 x g (8,000 rpm) for 5 minutes at room temperature. Remove supernatant completely and resuspend cells in 200 µl cold TE (not provided with kit).

2. Add 400 µl of Digestion Solution to 200 µl sample. Mix well. Add 3 µl of Proteinase K solution (2mg/150 µl) to sample and incubate at 55°C for 5 minutes.

Note: Do not add proteinase K solution directly to Digestion Solution.
Incubation period depends on the nature of sample.
If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml, not provided with kit), mix by vortexing, and incubate for 5 min at room temperature before continuing with step 3.

3. Add 260 µl of 100% ethanol, and mix well. Apply the mixture onto an MQ spin column that is placed in a 2.0 ml Collection Tube. Spin at 8,000 x g (10,000 rpm) for 2 minutes.

4. Discard the flow-through in the collection tube. Add 500 µl of Wash Solution, and spin at 8,000 x g (10,000 rpm) for 2 minutes.

5. Repeat Step 4.

6. Discard flow-through. Spin at 8,000 x g (10,000 rpm) for an additional minute to remove residual amount of Wash Solution.

7. Place the MQ column into a clean 1.5 ml microcentrifuge tube. Add 30-50 µl Elution Buffer into the center part of membrane in the column. Incubate at RT for 2 or 3 minutes. Incubating the tube at 37°C or 50°C for 2 minutes may increase recovery yield.

8. Spin at 8,000 x g (10,000 rpm) for 2 minute to elute DNA from the column.

9. For long term storage, keep aliquots of purified genomic DNA at -20°C.

Measure DNA quantity by UV absorption at A260 (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytical 0.7% agarose gel. The length of genomic DNA is around 50 kb.

Troubleshooting Guide

1. Low yield

a. *Improper storage of starting material*
>>>Prepare fresh samples and use immediately.
b. *Too much or too little starting material*
>>>Reduce or increase starting material accordingly.
c. *Incorrect preparation of buffers*
>>>Each step has to be strictly followed.

2. RNA contamination

Perform optional RNase treatment according to the protocol.

3. OD 260nm/280nm ratio outside 1.6-2.2 range

If the ratio of OD 260nm/280nm is greater than 2.2, there may be traces of ethanol present. If the ratio of OD 260nm/280nm is smaller than 1.6, there may be protein contamination. Make sure the sample is mixed well after proteinase K digestion.

4. DNA does not perform well

a. DNA Shearing
>>>Avoid repeated freezing and thawing of starting material; if samples are too old, start with a new sample.
b. Ethanol Carryover
>>>Spin additional steps before elution.