

MQ Fungal Genomic DNA Extraction Kit

Cat No. FDE-M-001-50

Size: 50 Preparations

MOLEQULE-ON[®]

Components	Quantity
Universal Digestion Buffer	12ml
Universal Buffer PF	6ml
Universal Buffer BD	12ml
Universal PW Solution	18ml
Universal Wash Solution	7.5ml
TE Buffer	10ml
Proteinase K	1.2ml
MQ Spin Columns and 2.0ml Collection Tubes	50 each

Preparations

- Universal Buffer BD contains chaotropic salt; avoid contact with skin and eyes.
- 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 7.5 ml Universal Wash Solution before use to obtain a working solution.

Storage

All the components of MQ Fungal Genomic DNA Extraction Kit should be stored dry at 15°C - 25°C. Proteinase K is supplied as 10 mg/ml solution. The solution can be kept at 4°C for 6 months, or -20°C for long-term.

Description

The kit provides a simple and convenient technique to isolate high quality DNA from Fungi 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. Purified genomic DNA ranges 20-50 kb in length can be obtained. Purified DNA is suitable for downstream applications such as Restriction Endonuclease Digestions, PCR, and other applications.

Features

- MQ Fungal Genomic DNA Extraction 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.

Materials Supplied By User

Microcentrifuge capable of at least 12,000 xg, Pipettes and pipette tips, Vortex mixer, Isopropanol, Ethanol (96-100%) RNase A (20 mg/ml, Optional for RNA-free DNA), Microcentrifuge tubes (1.5 ml or 2.0ml), water bath

Before Starting

This protocol is designed for purification of total DNA from fungi. 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 Fungal Genomic 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.

- **Proteinase K** is supplied in a ready-to-use solution form, but RNase A is not provided in this kit. If RNA-free DNA is required, please prepare RNA solution and follow procedures for RNA removal step.
- Check the Universal Digestion Buffer and Universal Buffer BD for salt precipitation before each use. If necessary, redissolve the precipitate by warming the solution at 56°C, then cool back down to room temperature before use.

- TE 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 water 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, add **22.5 ml ethanol** to 7.5 ml Universal Wash Solution respectively. Preheat the water bath or rocking platform to 56°C.

Procedure

1. Grind cell pellets collected from 0.1~3 ml fungi culture by centrifugation or 100-500 mg (wet weight) mycelia/spores in liquid nitrogen using a pestle. Transfer grinded sample to a clean 1.5 ml microcentrifuge tube.
2. Add 180 µl Universal Digestion Buffer and 20 µl Proteinase K to the sample, and mix thoroughly by vortexing. Incubate at 56°C for 30-60 min.
Note: If RNA-free genomic DNA is required, add 20 µl RNase A (20 mg/ml, not provided in the kit). Mix by vortexing, and incubate for 2 min at room temperature before continuing with step 3.
3. Add 100 µl Universal Buffer PF, mix by inverting, and 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. Add 200 Universal Buffer BD, mixes thoroughly by vortexing.
Note: If a gelatinous material appears at this step, incubate at 70°C for 10 min.
6. 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.
7. Transfer the mixture from step 6 (including any precipitate) into MQ column placed in a 2 ml collection tube. Centrifuge at 9,000 x g (12,000 rpm) for 1 min. Discard the flow-through.
8. Add 500 µl Universal PW Solution, and centrifuge for 1 min 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.
9. Add 500 µl Universal Wash Solution, and centrifuge for 1 min 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.
10. Place the empty column in the microcentrifuge and centrifuge for an additional 2 min 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 microcentrifuge 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.
11. Add 50-100 µl Buffer TE directly onto the center part of membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at 9,000 x g (12,000 rpm) to elute the DNA.

Notes:

- Warm the Buffer TE to 60°C will increase the elution efficiency.
- Elution with more than 100 µl (e.g. 200 µl) increases the DNA yield, but the concentration will be lower.
- For maximum DNA yield, repeat elution once as described in this step.
- A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate.
- For maximum DNA concentration, use the eluate in the microcentrifuge tube for the second elution step.