MQ Plant Genomic DNA Extraction Kit

Cat No. PGE-M-001-100
Size: 50 preps

MOLEQULE-ON®

Components	PGE-M-001-100 (100 Preps)
Buffer PCB	80 ml
Buffer BD	60 ml
PW Solution*	36 ml
Wash Solution*	15 ml
TE Buffer (pH 8.0)	20 ml
MQ Spin Column with 2.0ml Collection Tubes	100

* PW Solution and Wash Solution are supplied as concentrates. Before using for the first me, add 24 ml isopropanol to 36 ml PW Solution, and 45ml ethanol to 15ml Wash Solution, respectively.

Description

MQ Plant Genomic DNA Extraction Kit provides a simple and convenient technique to isolate high quality DNA from plants using a rapid spin-column format. DNA of cell lysates is selectively bound to the spin column and other impurities such as proteins and salts do not bind to the column and are eliminated in flow through. No phenol extraction and ethanol precipitations are required. The kit is also suitable for isolation of bacterial genomic DNA from colonies on dish. Purified genomic DNA is 20-50 kb in length. Purified DNA is suitable for downstream applications such as Restriction Endonuclease Digestions, PCR, and other applications.

Features

- Fast and easy. Processing uses a rapid spin-column format. The entire procedure takes approx. 30 minutes.
- Versatile. Various plant species have been tested.
- High quality of DNA. OD_{260}/OD_{280} of purified DNA is generally 1.7-1.9.
- The purified DNA is ready-to-use for most downstream applications.

Storage

MQ spin columns and all buffers should be stored dry, at room temperature (15-25°C) and are stable for 1 year under these conditions.

Materials Supplied by User

- Microcentrifuge capable of at least 12,000 × g
- Pipette Tips
- Vortexer
- Isopropanol
- β-mercaptoethanol
- Ethanol (96-100%)
- RNase A (20 mg/ml, Optional for RNA-free DNA)
- Microcentrifuge tubes (1.5 ml or 2 ml)
- Water bath for heating at 65°C

Safety Instructions

Buffer PCB and Buffer BD are harmful in contact with skin if swallowed, please avoid contact with eyes, skin, and clothes. Wash thoroughly after handling and see a doctor if necessary.

Before Starting

- This protocol is designed for extraction of total DNA from plant. 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. The MQ Plant 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.
- Check the Buffer PCB and Buffer BD for salt precipitation before each use. If necessary, re-dissolve the precipitate by warming the solution at 65°C, then cool back down to room temperature before use.
- TE Buffer is 10 mM Tris-HCl, 1 mM EDTA, pH 8.0. Water can be used as elutant in the final step if EDTA must be avoided for downstream applications, but it is not recommended if the pH of water is less than 7.0.
- PW Solution and Wash Solution are supplied as concentrates. Before using for the first time, add 24 ml isopropanol to 36 ml PW Solution, and 45ml ethanol to 15ml Wash solution, respectively.
- Preheat the water bath or rocking platform to 65°C

Protocol

1. Grind 100 mg fresh plant tissue (or 20 mg dry plant tissue) to fine powder in liquid nitrogen. Transfer the powder to a 1.5 ml tube.

2. Add 600 µl Buffer PCB and 12 µl of β-mercaptoethanol to the sample, and mix thoroughly by vortexing. Incubate at 65°C for 25 minutes.

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 before continuing with step 3.

3. Add 0.6 ml of chloroform to the tube, mix well by inverting 10 times. Centrifuge at 12,000 x g for 2 minutes. Carefully transfer the supernatant (400 µl) to a clean 1.5 ml tube.

4. Add 200 µl Buffer BD, mix thoroughly by vortexing. Incubate at 70°C for 10 minutes.

5. Add 200ul ethanol (96-100%), mix thoroughly by vortexing.

NOTE: If a gelatinous material appears at this step, vigorously shaking or vortexing is recommended.

6. Transfer the mixture from step 5 (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.

7. Add 500 µl PW Solution, and centrifuge for 1 minute at 9,000 x g (12,000 rpm).

NOTE: Check the label to ensure PW Solution was diluted with isopropanol.

8. Add 500 µl Wash Solution, and centrifuge for 1 minute at 9,000 x g (12,000 rpm).

NOTE: Discard the flow-through.

9. 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 column 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 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.

10. Add 50-100 µl TE Buffer directly onto the center part of MQ Column 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: Warming the TE Buffer to 60°C will increase the elution efficiency.
NOTE 2: For maximum DNA yield, repeat elution once as described in this step.
NOTE 3: To maximize DNA concentration, use the eluate in the microcentrifuge tube for the second elution step.