Plant Genomic DNA
SK8262

BIO BASIC
Quality-Affordable Research

Kit Contents

Components	B518261-0050 50 Preps	SK8262 100 Preps
Buffer PCB	40 ml	80 ml
Buffer BD	30 ml	60 ml
PW Solution	18 ml	36 ml
Wash Solution	8 ml	15 ml
TE Buffer (pH 8.0)	10 ml	20 ml
EZ-10 Column & Collection Tube	50	100
Protocol	1	1

Storage

EZ-10 columns and all buffers should be stored dry, at room temperature (15-25°C). The kit is stable for 2 years under these conditions.

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 doctor if necessary.

Introduction

The kit provides a simple and convenient technique to isolate high quality DNA from plants 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.The kit is also

Other Kits Available

EZ-10 Spin Column Plasmid DNA MiniPreps Kit
BS413 (50preps), BS414 (100preps), BS614 (250preps)

Plasmid DNA Extraction MAXI Prep Kit
BS4654 (4preps), BS466 (20preps)

ONE-4-ALL Genomic DNA Miniprep Kit
BS88503 (50preps), BS88504 (100preps),
BS88505 (250preps)

EZ-10 DNAaway RNA Miniprep Kit
BS88133 (50preps), BS88136 (250Preps)

Note 3: A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate.
Note 4: To maximize DNA concentration, use the eluate in the microcentrifuge tube for the second elution step.

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 using a rapid spin-column format. The entire procedure takes approx. 30 minutes.
- Versatile. Various plant species were 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.

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

Before Starting

This protocol is designed for purification 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. EZ-10 Column Plant 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.

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 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.

PW Solution and Wash Solution are supplied as concentrates. Before using for the first time, add 12ml ispropoanol to 18 ml PW solution in B518261-0050 kit (50 preps), or 24 ml isopropanol to 36 ml PW Solution in SK8262 (100 preps); Add 24ml of ethanol to 8ml Wash Solution B518261-0050 kit (50 preps), or 45ml ethanol to 15ml Wash Solution in SK8262 (100 preps). Preheat the water bath or rocking platform to 65°C.

Procedures

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 min.**

Note: If RNA-free genomic DNA is required, add 20 µl RNase A (20 mg/ml), mix by vortexing, and incubate for 2 min 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.**

Note: If a gelatinous material appears at this step, incubate at 70°C for 10 min.

5. 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.

6. Transfer the mixture from step 5 (including any precipitate) into the EZ-10 column placed in a 2 ml collection tube. Centrifuge at 9,000 x *g* (12,000 *rpm*) for 1 min. Discard the flow-through.

7. Add 500 µl 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 PW Solution was diluted with isopropanol.

8. Add 500 µl 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 Wash Solution was diluted with ethanol.

9. 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 EZ-10 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 EZ-10 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 EZ-10 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.

Note 1: Warm 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.