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BIO BASIC INC.

EZ-500 Spin Column Plant Genomic DNA Maxi-Preps Kit

PT92035 and PT92036

Version 6.0
ISO9001 Certified

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EZ-500 Spin Column Plant Genomic DNA Maxi-Preps Kit

Product information for PT92035/PT92036:

Kit Contents

Components	PT92035 4 Preps	PT92036 20 Preps
Buffer PCB	50 ml	250 ml
Universal Buffer BD	40 ml	200 ml
Universal PW Solution	18 ml	90 ml
Universal Wash Solution	7.5 ml	37.5 ml
TE Buffer	15 ml	75 ml
EZ-500 Column & 50 ml Collection Tube	4	20
Protocol	1	1

Note 1: Universal 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 **30 ml ethanol (96-100%)** to **7.5 ml Universal Wash Solution** before use to obtain a working solution.

Storage and Stability

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

Introduction

The kit is designed for a simple and large scale isolation of high molecular weight quality genomic DNA from a wide variety of plant species and tissue types including some very recalcitrant specimens. Samples may be fresh, frozen, or dried. DNA in plant tissues & cells is selectively absorbed on EZ-500 maxi-preps spin column, and other impurities such as proteins, salts do not bind to the column and are washed away. Up to 50 µg of plant DNA can be obtained by each preps. DNA purified by this procedure is around 20 kb. Purified genomic DNA can be used for PCR, Real Time PCR and other downstream applications including Southern Blotting, RAPD, RFLP and AFLP.

Features

- ü **Fast and Simple**. Using a rapid spin-column format. The entire procedure takes approx. 30 minutes.
- ü **High Purity of DNA**. OD_{260}/OD_{280} of purified DNA is generally >1.8.
- ü **Compatible** with many downstream applications such as PCR, restriction digestions, real-time PCR, multiplex PCR, RAPD, RFLP, AFLP, Southern Blotting and microsatellite analysis.
- ü **Suitable** for a wide variety of plant species and tissue types including some very recalcitrant specimens.

Materials Supplied by User:

Microcentrifuge capable of at least 12,000 × *g*
Pipets and pipet tips
Vortexer
Isopropanol
Ethanol (96-100%)
RNase A (20 mg/ml, Optional for RNA-free DNA)
Microcentrifuge tubes (50 ml)
Water bath for heating at 56°C

Procedures

1. Grind 2 g fresh plant tissue (or 1 g dry plant tissue) to fine powder in liquid nitrogen, transfer the powder to a 50 ml tube.

2. Add 10 ml Buffer PCB and 0.4 ml of β-mercaptoethanol to the sample, and mix thoroughly by vortexing. Incubate at 65°C for 50 minutes.

   Note: If RNA-free genomic DNA is required, add 0.5 ml RNase A (20 mg/ml), Mix by vortexing, and incubate for 2 min at room temperature before continuing with step 3.

3. Add 10 ml of chloroform to the tube, mix well by inverting 10 times. Centrifuge at 9,000 x g for 10 minutes. Carefully transfer the supernatant (8-9 ml) to a clean 50 ml tube.

4. Centrifuge at 9,000 *x g* for 5 minutes at room temperature. Transfer the supernatant to a new 50 ml tube.

5. Add equal volume of Universal Buffer BD, mix

thoroughly by vortexing.

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

6. Add equal volume of ethanol (96-100%), mix thoroughly.

7. Transfer the mixture from step 6 (including any precipitate) into the EZ-500 spin column placed in a 50 ml collection tube. Centrifuge at 9,000 *x g* for 1 minute. Discard the flow-through.

Note: If the column cannot hold entire supernatant. Please apply in multiple times. Spin briefly (1-2 minutes) at 9,000 x g between each application. After the final application of entire content in the column, spin at 9,000 x g for 3-5 minutes.

8. Add 5 ml Universal PW Solution, and centrifuge for 1 minute at 9,000 *x g*. Discard the flow-through.

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

9. Add 5 ml Universal Wash Solution, and centrifuge for 1 minute at 9,000 *x g*. Discard the flow-through.

   Note: Check the label to ensure Universal Wash Solution was diluted with ethanol.

10. Centrifuge for an additional 2 minutes at 9,000 *x g* to dry the membrane. Discard flow-through and transfer the EZ-500 spin column to a clean 50 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.

11. Add 1 ml TE Buffer directly onto the center part of membrane. Incubate at room temperature for 1 minute, and then centrifuge for 1 minute at 9,000 *x g* 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.

    Note 3: A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate.

    Note 4: For maximun DNA concentration, use the eluate in the microcentrifuge tube for the second elution step.

Other Kits Available

EZ-10 Spin Column Plasmid DNA MiniPreps Kit

BS413 (50 Preps)
BS414 (100 Preps)
BS614 (250 Preps)

EZ-10 Spin Column PCR Products Purification Kit

BS363 (50 Preps)
BS364 (100 Preps)
BS664 (250 Preps)

EZ-10 Spin Column DNA Gel Extraction Kit

BS353 (50 Preps)
BS354 (100 Preps)
BS654 (250 Preps)

PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY!
NOT INTENDED FOR HUMAN OR ANIMAL USE!

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