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BIO BASIC INC.

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

PT92037

Version 4.0
ISO9001 Certified

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

Product information for PT92037:

Kit Contents

Components	PT92037, 4 Preps
Universal Digestion Buffer	50 ml
Universal Buffer PF	25 ml
Universal Buffer BD	50 ml
Universal PW Solution	18 ml
Universal Wash Solution	7.5 ml
TE Buffer (pH 8.0)	10 ml
Proteinase K	5 ml
EZ-500 Spin Columns	4
50-ml Collection Tube	4
Protocol	1

Note 1: Universal Universal Buffer BD contains 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 **22.5 ml ethanol (96-100%)** for **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. Proteinase K is supplied as 10 mg/ml solution, the solution can be stored at 4°C for 6 months, or at -20°C for long term

Introduction

The kit provides a simple, rapid and efficient method for genomic DNA isolation in a large scale from filamentous fungal species including *S.cerevisiae*, *S. pomb*, *Pichia pastoris* using a rapid spin-column format. DNA from cell lysate is selectively absorbed in the column while other impurities are eliminated. Average DNA yield is around 10-100ug per preparation. Average size of the genomic DNA purified by this procedure is around 20-50 kb. No phenol/chloroform extraction, no ethanol precipitation and no liquid nitrogen for homogenizing sample are required. Purified fungi (yeast) genomic DNA can be used for PCR, SNP, RAPD, RFLP, AFLP, Southern blotting, transformation and other applications.

Features:

- ü **Fast and Simple.** Using a rapid spin-column format.
- ü **High Quality of DNA.** OD_{260}/OD_{280} ratio of purified fungal genomic DNA is generally >1.8. Purified DNA can be used for many downstream applications. Average size of the genomic DNA purified by this procedure is around 20-50 kb.
- ü **Suitable** for various filamentous fungal species including *Saccharomyces cerevisiae, Schizosaccharomyces pomb, Pichia pastoris, Candida albicans, Aspergillus niger, Collectotrichum musae, Fusarium oxysporum*.

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 cell pellets collected from 30-50 ml fungi culture by centrifugation or 3-5 g (wet weight) mycelia/spores in liquid nitrogen using a pestle. Transfer the sample to a clean 50 ml centrifuge tube.

2. Add 9 ml Buffer Digestion and 1 ml Proteinase K to the sample. Mix thoroughly by vortexing. Incubate at 56°C for 30-60 minutes.

Note: If RNA-free genomic DNA is required, add 1 ml RNase A (20 mg/ml). Mix by vortexing, and incubate for 2 min at room temperature before continuing with step 3.

3. Add 5 ml 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 50 ml tube.

5. Add 10 ml Buffer BD, mix thoroughly by vortexing.

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

6. Add 10 ml 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* (12,000 *rpm*) 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 4,000 x g between each application. After the final application of entire content in the column, spin at 4,000 x g for 3-5 minutes.

8. Add 5 ml PW Solution, and centrifuge for 1 minute 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 5 ml Wash Solution, and centrifuge for 1 minute 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. Centrifuge for an additional 2 minutes at 9,000 *x g* (12,000 *rpm*) 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 Buffer TE 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* (12,000 *rpm*) to elute the DNA.

    Note 1: Warm the Buffer TE 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 elute.

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

Other Kits Available

EZ-10 Spin Column Plasmid DNA Mini-Preps 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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