EZ-10 Spin Column Fungal Genomic DNA Mini-Preps Kit

Product information for FT82012:

Kit Contents

Components	FT82012 50 Preps
EZ-10 Column	50
2.0 ml Collection Tube	50
Universal Digestion Buffer	12 ml
Universal Buffer PF	6 ml
Universal Buffer BD	12 ml
Universal PW Solution (concentrate)	18 ml
Universal Wash Solution (concentrate)	7.5 ml
TE Buffer	10 ml
Proteinase K (10mg/ml)	1.2 ml
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

Transportation at ambient temperature. EZ-10 columns and all buffers should be stored dry, at room temperature (15-25°C). Columns and buffers are stable for 1 year. 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.

Introduction

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. The kit is also suitable for isolation of bacterial genomic DNA from colonies on dish. Purified genomic DNA ranges 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 approximately 30 minutes.
- ✓ High quality of DNA. $OD_{260/280}$ of purified DNA is generally > 1.8.
- ✓ No phenol/chloroform extraction or ethanol precipitation is required.

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 (1.5 ml or 2 ml)
Water bath for heating at 56°C

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. EZ-10 Column fungal 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.

- ✓ **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 **Buffer Digestion** 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.
- ✓ CE 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.

Procedures

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 microtube.

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 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.

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 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.

11. Add 50-100 µl Buffer TE 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 Buffer TE to 60°C will increase the elution efficiency.

Note 2: Elution with more than 100 µl (e.g. 200 µl) increases the DNA yield, but the concentration will be lower.

Note 3: For maximum DNA yield, repeat elution once as described in this step.

Note 4: A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate.

Note 5: For maximum DNA concentration, use the eluate in the microcentrifuge tube for the second elution step.