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BIO BASIC INC.

EZ-10 Column Yeast Genomic DNA Purification Kit

BS8257

Version 6.1
ISO9001 Certified

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EZ-10 Column Yeast Genomic DNA Purification Kit

Kit Content

Components	BS8257, 50 Preps
Universal Digestion Buffer	12 ml
Universal Buffer PY	6 ml
Universal Buffer BD	12 ml
Universal PW Solution	18 ml
Universal Wash Solution	7.5 ml
CE Buffer (pH 9.0)	15 ml
Proteinase K	1.2 ml
Snailase Reaction Buffer	75 ml
Snailase Storage Buffer	5 ml
Snailase	600 mg
EZ-10 Spin Column	50
2 ml Collection Tube	50
Protocol	1

Note 1: Buffer BD contains a chaotropic salt; avoid contact with skin and eyes.

Note 2: PW Solution and Wash Solution are supplied as concentrates. Add **12 ml isopropanol** to 18 ml **PW Solution** and **22.5 ml ethanol** (96-100%) for 7.5 ml **Wash Solution** before use to obtain a working solution.

Storage and Stability

EZ-10 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 kept at Room Temperature for 6 months, for long-term storage keep at -20 °C.

Introduction

The kit provides a simple and convenient technique to isolate high quality DNA from yeast 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 yeast 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

1. Fast and easy processing using a rapid spin-column format. The entire procedure takes approx 30 minutes.
2. High yield. 5-20 µg of yeast genomic DNA can be obtained from 1 ml (10^8-10^9 cells) of overnight culture.
3. High quality of DNA. $OD_{260/280}$ of purified DNA is generally1.7-1.9.
4. No phenol/chloroform extraction or ethanol precipitation is required.

Reagents and Materials Provided By User

Microcentrifuge capable of at least 12,000 × *g*
Pipets and pipet tips
Vortexer
Isopropanol
Ethanol (96-100%)
RNase A (10 mg/ml, Optional for RNA-free DNA)
Water bath for heating at 56°C

Before Starting

This protocol is designed for purification of total DNA from yeast. 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 Yeast 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.

Snailase working stock: Add Snailase into the Snailase Storage Buffer, mix well by gently inverting, then store at -20ºC for use.
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 RNase solution and see protocol to add the RNA removal step.

Check the **Buffer Digestion** and **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 elution 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 **12 ml isopropanol** to 18 ml PW Solution, **22.5 ml ethanol** to 7.5 ml Wash Solution, respectively. Preheat the water bath or rocking platform to 56 °C.

Protocol

1. Collect 1.0 ml of yeast culture (1×10^7 cells) in a 1.5 ml microcentrifuge tube and centrifuge at 10,000 x *g* for 30 seconds. Discard the supernatant completely.

2. Add 600 µl of Snailase Reaction Buffer, 1.2 µl of β-mercaptoethanol and 50 µl of Snailase working stock per 20 mg wet weight yeast in the 1.5 ml tube. Incubate at 37 ºC for 3 hours. Invert the tube occasionally. If lyticase was used, please use 50 µl of Lyticase Enzymatic Storage Buffer (containing 300 U or more) per 20 mg wet weight yeast. Centrifuge at 4,500 x *g* for 10 minutes, discard the supernatant.

3. Add 180 µl Buffer Digestion 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), mix by vortexing, and incubate for 2 min at room temperature before continuing with step 4.

4. Add 100 µl Buffer PY, mix by inverting, incubate at -20 °C for 5 minutes.

5. Centrifuge at 12,000 x *g* for 5 minutes at room temperature. Transfer the supernatant to a new 1.5 ml tube.

6. Add 200 Buffer BD, mix thoroughly by vortexing.
   Note: If a gelatinous material appears at this step, incubate at 70 °C for 10 min.

7. 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.

8. Transfer the mixture from step 7 (including any precipitate) into the EZ-10 column placed in a 2 ml collection tube. Centrifuge at 9,000 x *g* for 1 min. Discard the flow-through.

9. Add 500 µl PW Solution, and centrifuge for 1 min at 9,000 x *g.* Discard the flow-through.
   Note: Check the label to ensure PW Solution was diluted with isopropanol.

10. Add 500 µl Wash Solution, and centrifuge for 1

min at 9,000 x *g*. Discard the flow-through.

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

11. Place the empty column in the microcentrifuge and centrifuge for an additional 2 min at 9,000 x *g* 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.

12. Add 50-100 µl Buffer CE 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* to elute the DNA.

Note 1: Warm the Buffer CE 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: For maximum DNA concentration, use the eluate in the microcentrifuge tube for the second elution step.

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NOT INTENDED FOR HUMAN OR ANIMAL USE!

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