TIANGEN®

TIANamp Yeast DNA Kit

For isolation of genomic DNA from yeast cells

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TIANamp Yeast DNA Kit

(Spin Column)

Cat.no. 4992449

Kit Contents

Contents	4992449 50 preps
Buffer GA	15 ml
Buffer GB	15 ml
Buffer GD	13 ml
Buffer PW	15 ml
Buffer TE	15 ml
Proteinase K	1 ml
Spin Columns CB3	50
Collection Tubes 2 ml	50
Handbook	1

Compatible Reagents

RNaseA (100 mg/ml)

Storage

TIANamp Yeast DNA Kit should be kept in dry place can be stored at room temperature (15-30°C) for up to 15 months without showing any reduction in performance and quality. If any precipitate forms in the buffers, it should be dissolved by warming the buffers at 37°C for 10 min before use.

Introduction

TIANamp Yeast DNA Kit is based on silica membrane technology and provided special buffer system for extraction of gDNA from yeast sample. The spin column is made of new type silica membrane which can bind DNA optimally on given salt and pH conditions. Simple centrifugation processing completely removes contaminants and enzyme inhibitors such as proteins and divalent cations. Purified DNA is eluted in low-salt buffer or water, ready for use in downstream applications.

DNA purified by TIANamp Yeast DNA Kit is highly suited for restriction analysis, PCR analysis, Southern blotting and cDNA library.

Concentration Measurement

Spectrophotometer or plate counting method can be adopted to determine the concentration of yeast cell suspension. For *S cerevisiae*, OD_{600} of 1 is roughly equal to a concentration of 1-2 × 10^7 cells/ml.

Important Notes

1. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size.
2. If a precipitate has formed in Buffer GA or Buffer GB, warm buffer to 37°C until the precipitate has fully dissolved.
3. All centrifugation steps should be carried out in a conventional table-top microcentrifuge at room temperature (15-30°C).

Materials required but not supplied

1. Lyticase (TIANGEN)
2. Sorbitol Buffer: 1.2 M Sorbitol in 0.1 M PBS solution (pH 7.4)
   0.1 M PBS solution (pH 7.4): 77.4 ml 0.1 mol/L Na_2HPO_4 + 22.6 ml 0.1 mol/L NaH_2PO_4

Protocol

Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

1. Pipet an appropriate amount of yeast cell (no more than 5 × 10^7 cells) in a microcentrifuge tube, centrifuge for 1 min at 12,000 rpm (~13,400 × g). Discard supernatant.

   Note: If the sample is more than 700 µl, repeat the centrifugation process to collect all yeast cells into one tube.
2. Lysis (enzyme method) to crack yeast cell wall: Add 600 µl Sorbitol Buffer and 50 U Lyticase (should be prepared by user). Mix thoroughly, and incubate at 30°C for 30 min. Centrifuge for 10 min at 4,000 rpm (~1500 × g). Discard supernatant.

   Note: Above protocol is optimized for processing 5 × 10^7 yeast cells. Lysis time and Lyticase concentration should be varied according to the type of strain and amount of yeast cell processed.
3. Add 200 µl Buffer GA to resuspend the cell pellet. Mix thoroughly by vortex.

   Note: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml, should be prepared by user). Mix by vortex for 15 sec, and incubate for 5 min at room temperature (15-30°C).
4. Add 20 µl Proteinase K. Mix thoroughly.
5. Add 220 µl Buffer GB to the sample, mix thoroughly by upsides and down, and incubate at 70°C for 10 min to yield a homogeneous solution. Briefly centrifuge the microcentrifuge tube to remove drops from the inside of the lid.

   Note: White precipitates may form when Buffer GB is added. They will not interfere with the procedure and will dissolve during the heat incubation at 70°C. If precipitates do not dissolve during heat incubation, it indicates that the cell is not completely lysed and may result in low yield of DNA and impurity in DNA.
6. Add 220 µl ethanol (96-100%) to the sample, and mix thoroughly by upsides and down. White precipitates may form on addition of ethanol. Centrifuge briefly to remove drops from the inside of the lid.
7. Pipet the mixture from step 6 into the Spin Column CB3 (in a 2 ml collection tube) and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard flow-through and place the spin column into the collection tube.

8. Add 500 µl Buffer GD **(Ensure ethanol (96-100%) has been added)** to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec, then discard the flow-through and place the spin column back to the collection tube.
9. Add 600 µl Buffer PW **(Ensure ethanol (96-100%) has been added)** to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and place the spin column back to the collection tube.
10. Repeat step 9.
11. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min to dry the membrane completely. Open lid of CB3 and stay at room temperature for a while to dry the membrane completely.

    Note: The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during DNA elution. Residual ethanol may interfere with down-stream reactions.
12. Place the Spin Column CB3 in a new clean 1.5 ml microcentrifuge tube, and pipet 50-200 µl Buffer TE or distilled water directly to the center of the membrane. Incubate at room temperature for 2-5 min, and then centrifuge for 2 min at 12,000 rpm (~13,400 × g).
13. Optional: For increased DNA concentration, add the solution obtained from step 12 to the center of membrane again. Incubate at room temperature for 2-5 min, and then centrifuge for 2 min at 12,000 rpm (~13,400 × g).

    Note: If the volume of eluted buffer is less than 50 µl, it may affect recovery efficiency. The pH value of eluted buffer will have some influence in eluting. We suggest choosing buffer TE or distilled water (pH 7.0-8.5) to elute gDNA. For long-term storage of DNA, eluting in Buffer TE and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.

Analysis of DNA concentration and purity

Size of gDNA extracted by this kit is related with the sample storage condition, shearing force during operation and some other factors. Purified DNA can be analysis by electrophoresis gel and UV-Spectrophotometer.

DNA has a significant peak at OD_{260}. An OD_{260} of 1 corresponds to a 50 μg/ml of dsDNA solution or a 40 μg/ml of ssDNA solution.

OD_{260}/OD_{280} ratio value should be within 1.7-1.9. If ddH_2O is used to elute DNA, the ratio value would be lower, since the pH value and the existence of ion could affect the absorption value.