CYDN29-CHANGYU Midi/Maxi Yeast Genomic DNA Kit

Catalog number	Package
CYDN2901	10 preps

Storage and shipping: 15-25 °C. Stable for 12 months at room temperature. At low ambient temperature some detergent components of the Yeast Lysis Buffer may separate out and cause turbidity or precipitation; warm in a 37 °C water bath for a few minutes with gentle swirling until the solution clears, and do not shake vigorously, to avoid excessive foaming. The Protein Precipitation Solution may also precipitate; warm at 37 °C for a few minutes to help redissolve; if it does not dissolve completely, the use of the solution is not affected and the upper phase can be used directly. Keep all solutions tightly capped after use to avoid evaporation, oxidation and pH drift.

Description

This kit is used for rapid extraction of genomic DNA from yeast. In the Yeast Lysis Buffer, formulated for the characteristics of yeast cells, the cells are lysed and release genomic DNA; the Protein Precipitation Solution then selectively precipitates and removes proteins; finally the purified genomic DNA is precipitated with isopropanol and redissolved in DNA Dissolution Solution.

Kit components

Component	Storage	10 preps (CYDN2901)
Yeast Lysis Buffer	15-25 °C	100 ml × 2
Protein Precipitation Solution	15-25 °C	70 ml
DNA Dissolution Solution	15-25 °C	30 ml

Features

No toxic phenol, chloroform or other reagents are required.

Fast and simple: the whole procedure can be completed within 1 hour.

Stable results and high yield; typical A260/A280 is 1.7-1.9 and the DNA length can reach 50 kb-150 kb. The DNA can be used directly for PCR, Southern blot, various restriction digestions and library construction.

❖ Procedure

1. Pipette 60-70 ml of yeast culture into a 100 ml centrifuge tube; centrifuge at 2,500 ×g for 2 minutes and discard the supernatant as completely as possible (aspirate with a pipette if necessary).

2. Vortex at high speed to break up and resuspend the yeast cell pellet.

3. Add 20 ml of Yeast Lysis Buffer and mix by vortexing, or mix by pipetting repeatedly with a 1 ml tip.

Resuspension and dispersion of the yeast cells is very important for the subsequent lysis step; the cells must be fully dispersed and resuspended.

4. Place the lysate in a 70 °C water bath for 15-30 minutes.

If the yield is low, the water bath temperature may be raised and the incubation time extended; vortex briefly several times during incubation to aid lysis.

5. Cool on ice for at least 5 minutes to return to room temperature.

6. Add 6.8 ml of Protein Precipitation Solution to the lysate at room temperature and vortex continuously at high speed on a vortexer for 25 seconds. Some small protein clumps may be seen after mixing. Incubate on ice for 5 minutes.

7. Centrifuge at 2,500 ×g (the centrifugal force may be increased if necessary) for 5 minutes. A protein pellet should be visible at the bottom of the tube, and some protein precipitate may also be seen floating on the surface of the liquid.

8. Carefully and slowly transfer the supernatant to a new 100 ml centrifuge tube; do not take up the precipitate.

When taking the supernatant, do not take up the protein precipitate at the bottom of the tube or floating on the surface. If protein precipitate is accidentally transferred to the new tube, centrifuge again for 2 minutes and take the supernatant.

9. Add an equal volume of room-temperature isopropanol and mix by inverting 30 times, or until a flocculent DNA precipitate (or a white cloudy precipitate) appears.

10. Centrifuge at 2,500 ×g for 5 minutes (the centrifugal force may be increased if necessary). A white DNA pellet is visible at the bottom of the tube; pour off the supernatant.

11. Add 20 ml of 70% ethanol, invert several times to rinse the DNA pellet, centrifuge at 2,500 ×g for 2 minutes and pour off the supernatant (take care not to pour out the DNA pellet). After inverting, tap the tube a few times on absorbent paper to drain residual ethanol; residual ethanol around the pellet at the bottom and on the tube wall can also be carefully aspirated with a tip, and the pellet is air-dried for a few minutes.

Do not over-dry the pellet, otherwise the DNA becomes extremely difficult to dissolve; too much residual ethanol must also be avoided, since ethanol may inhibit downstream reactions such as restriction digestion.

12. Add 1-3 ml of DNA Dissolution Solution to rehydrate and dissolve the DNA pellet, mix by flicking the tube wall, and incubate at 65 °C for 30-60 minutes (do not exceed one hour), or leave at room temperature or 4 °C overnight to rehydrate the DNA. Flick the tube wall occasionally during this period to help rehydration.

13. Add 0.5-1 ml of RNase A (10 mg/ml), mix by inversion and incubate at 37 °C for 30-60 minutes to remove residual RNA.

This step mainly removes residual RNA; if a large amount of RNA remains, the incubation may be extended or the amount of RNase A increased. If residual RNA does not affect the experiment, this step may be omitted. If residual RNase may affect the experiment, the DNA can also be extracted with an equal volume of phenol/chloroform and recovered by standard ethanol precipitation.

14. The DNA can be stored at 2-8 °C; for long-term storage, store at -20 °C.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.