CYDN28-CHANGYU Yeast Genomic DNA Kit (Solution Type)

Catalog number	Package
CYDN2801	50 preps
CYDN2802	100 preps
CYDN2803	200 preps

Storage and shipping: 15-25 °C. Stable for 12 months at room temperature.

Description

This kit is designed for rapid extraction of genomic DNA from yeast. Yeast Lysis Buffer, formulated for the characteristics of yeast cells, lyses the cells and releases genomic DNA. Protein Precipitation Buffer then selectively precipitates and removes protein, and purified genomic DNA is precipitated with isopropanol and redissolved in DNA Dissolution Buffer.

Kit components

Component	Storage	50 preps (CYDN2801)	100 preps (CYDN2802)	200 preps (CYDN2803)
Yeast Lysis Buffer	15-25 °C	15 ml	30 ml	60 ml
Protein Precipitation Buffer	15-25 °C	5 ml	10 ml	20 ml
DNA Dissolution Buffer	15-25 °C	5 ml	5 ml	10 ml

The kit is stable for 12 months at room temperature.

Storage notes

At low ambient temperature, some detergent components of the Yeast Lysis Buffer may precipitate, making the buffer turbid. Warm in a 37 °C water bath for a few minutes with gentle swirling until it becomes clear; do not shake vigorously, as excessive foam may form.

Protein Precipitation Buffer may precipitate. Warm in a 37 °C water bath for a few minutes to help redissolve; if it does not dissolve completely, the buffer is still usable - simply take the upper solution.

Avoid prolonged exposure of reagents to air, which causes evaporation, oxidation and pH changes. Keep all solutions tightly capped after use.

Features

No toxic reagents such as phenol or chloroform are required.

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

Stable results and high yield (more than double the yield of spin-column formats), with a typical OD260/OD280 of 1.7-1.9 and a length of 50 kb-150 kb; the DNA is suitable for PCR, Southern-blot, restriction digestion and library construction.

❖ Procedure

1. Pipette 1.5 ml yeast culture into a 1.5 ml centrifuge tube; centrifuge at 12,000 rpm for 2 minutes and discard the supernatant as completely as possible, using a pipette if necessary.

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

3. Add 300 µl Yeast Lysis Buffer, vortex to mix, or mix by repeated pipetting with a 1 ml pipette tip.

Resuspending and dispersing the yeast cells is very important for the next 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 increased and the time extended; vortexing several times during incubation helps lysis.

5. Place on ice for at least 5 minutes to cool to room temperature.

6. Add 100 µl Protein Precipitation Buffer to the lysate at room temperature and vortex continuously at high speed for 20 seconds; small protein clumps may be visible after mixing. Incubate on ice for 5 minutes.

7. Centrifuge at 13,000 rpm for 5-10 minutes. A protein pellet should be visible at the bottom of the tube, and some protein may float on the surface.

8. Carefully and slowly transfer the supernatant to a new 1.5 ml centrifuge tube without disturbing the pellet.

Note: When transferring the supernatant, do not disturb the protein pellet at the bottom or the material floating on the surface. If protein is accidentally transferred, centrifuge again for 2 minutes and take the supernatant.

9. Add an equal volume of room-temperature isopropanol (about 400 µl) and invert 30 times, or until a flocculent DNA precipitate (or white cloudy precipitate) appears.

10. Centrifuge at 12,000 rpm for 1 minute. A white DNA pellet should be visible at the bottom of the tube; discard the supernatant.

11. Add 1 ml 70% ethanol, invert several times to rinse the DNA pellet, centrifuge at 12,000 rpm for 1 minute and discard the supernatant (take care not to discard the DNA pellet). Invert the tube, tap it on absorbent paper to drain residual ethanol, remove residual ethanol around the pellet and on the tube wall with a pipette tip if necessary, and air-dry the pellet for several minutes.

Note: Do not over-dry the pellet, or the DNA will be very difficult to dissolve. Do not leave too much ethanol either, as residual ethanol may inhibit downstream reactions such as restriction digestion.

12. Add 40 µl DNA Dissolution Buffer to rehydrate and dissolve the DNA pellet. Mix by flicking the tube. Incubate at 65 °C for 30-60 minutes (no more than 1 hour), or leave at room temperature or 4 °C overnight, flicking the tube occasionally to help rehydration.

13. Add 10-15 µl RNase A (10 mg/ml), or 1-2 µl RNase A (100 mg/ml), mix by inversion and incubate at 37 °C for 30-60 minutes to remove residual RNA.

This step removes residual RNA. If residual RNA is abundant, the incubation may be extended. If residual RNA does not affect the experiment, this step may be omitted. If the residual RNase may affect the experiment, the RNA may instead be removed by extraction with an equal volume of phenol/chloroform followed by standard ethanol precipitation to recover the DNA.

Store the DNA at 2-8 °C; for long-term storage, keep at -20 °C.

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