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Yeast Genomic DNA Extraction Kit

Cat No.: D1900

Package: 50T/ 100T

Storage: Store in dry and temperate condition(15℃-25℃), re-test period for 12 months.

Kit Contents：

Component	D1900-50T	D1900-100T	Storage
RNase A	1ml	1ml×2	-20℃
Proteinase K	1ml	1ml×2	-20℃
Lysing Enzymes	1.25	2.5ml	-20℃
β-Mercaptoethanol	300μl	600μl	2℃-8℃
Sorbitol Buffer	25ml	50ml	RT
Buffer A	10ml	20ml	RT
Buffer B	10ml	20ml	RT
Washing buffer	15ml	15ml×2	RT
Elution buffer	10ml	30ml	RT
Adsorption Column	50 units	100 units	RT
Collection tube	50 units	100 units	RT

Product Description

Yeast Genomic DNA Extraction Kit is based on silica membrane technology and provided special buffer system for extraction of genomic DNA from yeast sample.The silicon matrix material used in the centrifugal adsorption column is a unique new material of the company, which can efficiently and specifically adsorb DNA, and can remove impurity proteins and other organic compounds in cells to the greatest extent. The extracted genomic DNA fragments are large, high in purity, and stable and reliable in quality. The genomic DNA extracted by this kit can be used in various routine operations, including restriction enzyme digestion, PCR, library construction, Southern hybridization and other experiments.

Protocol

Note: Please add Absolute Ethanol to the Washing Buffer before use. Please refer to the label on the bottle for the added volume. Unless otherwise specified, all centrifugation steps are centrifuged at room temperature using a benchtop centrifuge.

1. Pellet 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). Remove the supernatant as much as possible.
2. Lysis to crack yeast cell wall: Add 470μl Sorbitol Buffer, completely resuspend the cell pellet. Add 25μl Lysing Enzymes and 5μl β-Mercaptoethanol. Mix thoroughly, and incubate at 30°C for 1-2 h. During digestion, the centrifuge tube can be inverted several times.

3. Centrifuge for 1min at 12,000 rpm . Discard supernatant.
4. Add 200µl Buffer A to resuspend the cell pellet. Mix thoroughly by vortex. Add 20µl RNase A, invert and gently rotate the tube several times, incubate at room temperature for 10min.
5. Add 20µl Proteinase K (10mg/ml). Mix thoroughly. Incubate at 65℃ for 15-30min. Invert the tube several times during incubating until the sample is completely digested.
6. Add 200µl Buffer B and 200µl ethanol (96-100%). Mix thoroughly. Flocculent precipitation may occur, which will not affect the extraction of DNA. Solution and precipitation both can be added to the Adsorption Column and incubate at room temperature for 2 minutes
7. Centrifuge for 2min at 12,000 rpm, discard flow-through, and re-use the collection tube in the next step.
8. Wash the Adsorption Column with 600µl Washing buffer(ensure that absolute ethanol has been added), centrifuge at 12,000rpm for 1min, discard the flow-through and re-use the collection tube in the next step.
9. Repeat wash step 8 with another 600µl Washing buffer.
10. Centrifuge the empty column at 12,000rpm for 2min. Incubate at room temperature or 50℃ to dry the column. It is critical for removing ethanol from the column.
11. Place column into a new clean centrifuge tube. Add 50-200µl Elution buffer onto the column matrix, incubate at room temperature for 5min. Centrifuge at 12,000rpm for 1 min.
12. To increase DNA concentration, add the solution obtained from step 11 to the center of membrane again, centrifuge at 12,000 rpm for 2 min.

Notes

1. Avoid repeated freezing and thawing of samples. Otherwise, the extracted DNA fragments are smaller and the extracted amount is also decreased.
2. If the precipitate occurs in the kit components, re-dissolved in 65℃ water bath before use, which will not affect the results.
3. If Adsorption Column is blocked , the time of centrifugation can be extended.
4. If the volume of elution buffer is less than 50µl, it may affect recovery efficiency. The pH value of elution buffer will have big influence in eluting. If using distilled water, pH should be controlled at 8.0 (adjusted by NaOH), below 7.0 will affect elution efficiency.
5. Detect the concentration and purity of DNA: The purity of plasmid DNA influenced by many factors, the DNA purity can be detected by Agarose gel electrophoresis and Ultraviolet spectrophotometer. DNA should have absorption peak in OD_{260}, OD_{260}=1 is equal to 50µl double-stranded DNA, 40µl single-stranded DNA. OD_{260}/OD_{280} should be 1.7-1.9, the value will be lower if using distilled water in eluting, but do not show the purity is low.

Related products

D1010 DNA Loading Buffer,6×

T1060 TAE Buffer,50×

T1050 TBE Buffer,5×

M1060　D2000 DNA Ladder

M1400　1kb DNA Ladder

G8142　GoldView II Nuclear Staining Dyes(5000×)

D1100　Plasmid Extraction Mini Kit