Genomic DNA Bulk Extraction Kit for yeast

CatNo.: D1910
Package: 50T/100T
Storage: Preserved at room temperature for a retest period of 12 months and even longer for 2-8℃. (Note: RNase A, proteinase K, yeast wall breaking enzyme are shipped in accessories and stored at-20℃)

Component	10T	Storage
RNase A	300μL×2	-20℃
Proteinase K	1mL×5	-20℃
The yeast wall-breaking enzyme	1.5mL×5	-20℃
Mercaptol reductant	1.5mL	2-8℃
Sorbitol Buffer	25mL×4	RT
Solution A	50mL	RT
Solution B	50mL	RT
Wash Buffer	15mL×2	RT
Elution Buffer	20mL	RT
Adsorption Column	10 个	RT
Collecting Pipe	20 个	RT
Instruction	1	

Product Description:

This kit uses a centrifugal adsorption column that can specifically bind DNA and a unique buffer system to extract yeast genomic DNA. The silicon matrix material used in the centrifugal adsorption column is the unique new material of the company, which can be highly efficient and specific DNA adsorption and maximize the removal of impurity proteins and other organic compounds in cells. The extracted genomic DNA fragments are large, of high purity and of stable and reliable quality. The genomic DNA extracted using this kit can be used for a variety of routine operations, including enzyme digestion, PCR, library construction, Southern hybridization, and other experiments.

Operation steps (for reference only):

Before use, add absolute ethanol to the rinse solution, and refer to the label on the bottle body (add 45mL of absolute ethanol separately for each bottle of rinse solution). All centrifugation steps were performed at room temperature using a benchtop centrifuge.

1、 Yeast cells were collected at 20-40mL (no more than 10^9 cells) in a centrifuge tube, centrifuged at 10000rpm for 1min, and remove the supernatant as far as possible.

2、 Break of the yeast cell wall: add 9mL sorbitol Buffer to the yeast body. Fully suspend the bacteria, add 600μL yeast wall breaking enzyme and 100μL sulfhydryl reducing agent, and fully mix well.30℃ for 1-2h, during which the inverted could was mixed several times.

3、 The sample was centrifuged for 1min at 10000rpm, the supernatant was discarded and the precipitate was collected.

4、 5mL of solution A was added to the precipitate, and 50μL of RNase A was added to the suspension, fully reversed and left at room temperature for 10min.

5、 Add 500μL of proteinase K and mix well and fully reversed. 65℃ water bath digestion for 30-60min, during

which the centrifugal tube can be mixed several times until the sample is fully digested.

6、 Add 5mL of solution B and 5mL of absolute ethanol and mix well. At this time, flocculent precipitation may occur, not affecting the extraction of DNA. Both solution and flocculent precipitation can be added to the adsorption column and placed at room temperature for 2min.

7、 After centrifugation at 10000rpm for 2min, the waste liquid was discarded and the adsorption column was placed into the collecting tube.

8、 Add 7mL of rinse to the adsorption column (check whether absolute ethanol has been added before use). After centrifugation at 10000rpm for 1min, the waste liquid was discarded and the adsorption column was placed into the collecting tube.

9、 7mL of rinsing solution was added to the adsorption column, centrifuged at 10000rpm for 1min, discard the waste solution, and the adsorption column was placed into the collecting tube.

10、 Centrifuge at 10000rpm for 2min and open the adsorption column at room temperature or a 50℃ temperature box for several minutes to remove the residual rinse solution from the adsorption column, otherwise the ethanol in the rinse would affect subsequent experiments such as enzyme digestion, PCR, etc.

11、 The adsorption column was placed in a clean centrifuge tube with 1-2mL of eluate preheated with a 65℃ water bath dropped to the center of the adsorption membrane, left at room temperature for 5min and centrifuged at 1min at 10000rpm.

12、 The eluate obtained by centrifugation was added to the adsorption column and centrifuged at 10000 rpm for 2 min to yield high quality genomic DNA.

Note:

1、 Samples should avoid repeated freezing and thawing, otherwise it will cause small extracted DNA fragments and decreased extraction amount.

2、 If the solution in the kit is precipitated, it can be redissolved in a 65℃ water bath before use, without affecting the extraction effect.

3、 If the centrifugation step in the experiment is blocked, the centrifugation time can be extended appropriately.

4、 The volume of the elution buffer should not be less than 1 mL, too small volume will affect the recovery efficiency; the pH value of the eluate also affects the elution efficiency, the pH value should be about 8.0 (NaOH the pH value of the water to this range), the pH value lower than 7. 0 will lower the elution efficiency. The DNA product should be stored at-20℃ to prevent DNA degradation.

5、 DNA concentration and purity detection: the size of the resulting genomic DNA fragments is related to the storage time of the sample, the shear force during the operation and other factors. The recovered DNA fragments can be detected for concentration and purity by agarose gel electrophoresis and UV spectrophotometer. The DNA should have a significant absorption peak at OD260, with an OD260 value of 1.0 equivalent to approximately 50 μg/mL double-stranded DNA, 40μg/mL single-stranded DNA. The OD260/ OD280 ratio should be 1.7-1.9. If the elution buffer is not used, while the deionized water is used, the ratio will be low, because the pH and the presence of ions will affect the light absorption value, but it does not indicate low purity.

Related products:

D1010 6×DNA Loading Buffer
T1060 50×TAE Buffer
T1050 5×TBE Buffer
M1060 D2000 DNA Ladder
M1400 1kb DNA Ladder
G8142 GoldView II Nucleic Acid Stain(5000×)
D1160 Yeast Plasmid Extraction Kit