V02

Yeast genome DNA extraction kit

Cat: D1900
Package: 50T/100T
Storage: RT, Valid for 1 year (RNase A, protease K and wall breaking enzyme are delivered in accessory form and stored at -20℃).

Product composition:

Kit composition	50T	100T	Storage
RNase A	100μL	100μL×2	-20℃
Protease K	1mL	1mL×2	-20℃
Yeast wall breaking enzyme	1.25mL	1.25mL ×2	-20℃
Sulfhydryl reducing agent	300μL	600μL	RT
Sorbitol Buffer	25mL	25mL×2	RT
Solution A	10mL	20mL	RT
Solution B	10mL	20mL	RT
Bleaching solution	15mL	15mL×2	RT
Eluate	15mL	30mL	RT
Adsorption column	50	100	
Collecting tube	50	100	
Specification	1	1	

Notes:

1. Please add anhydrous ethanol to the bleach solution before use, and add the volume according to the label on the bottle (each bottle needs to add 45mL anhydrous ethanol separately).
2. All centrifugation steps are performed using a table centrifuge at room temperature.

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 our company's unique new material, which can efficiently and specifically adsorb DNA, and can maximize the removal of foreign proteins and other organic compounds in the cell. The extracted genomic DNA fragments are large, high purity, stable and reliable. 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:

1. Yeast cells (no more than 5×10^7cells) were taken, centrifuged at 12000rpm for 1min, and the supernatant was removed as far as possible.
2. Yeast cell wall breaking: 470μL sorbitol Buffer was added to the yeast body. Fully suspended bacteria, adding 25μL yeast wall breaking enzyme and 5μL sulfhydryl reducing agent, thoroughly mixed. Treatment at 30℃ for 1-2h, during which the centrifugal tube can be reversed and mixed several times.
3. Centrifuge at 12000rpm for 1min, discard the supernatant, and collect the precipitation.
4. Add 200μL solution A to the precipitation, fully suspend precipitation, add 2μL RNase A to the suspension, fully invert and mix, and place at room temperature for 10min.
5. Add 20μL of protease K and mix thoroughly upside down. Digestion in a water bath at 65℃ for 15-30min. During digestion, the centrifuge tube can be reversed and mixed several times until the sample is completely digested.
6. Add 200μL solution B, then add 200μL anhydrous ethanol, and mix thoroughly upside down. Flocculation precipitation may occur at this time, which will not affect the extraction of DNA. Both solution and

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flocculation precipitation can be added to the adsorption column and placed at room temperature for 2min.

7. Centrifuge at 12000rpm for 2min, discard the waste liquid, and put the adsorption column into the collection tube.
8. Add 600μL bleach solution to the adsorption column (check whether anhydrous ethanol has been added before use). Centrifuge at 12000rpm for 1min, discard the waste liquid, and put the adsorption column into the collection tube.
9. Add 600μL bleach solution to the adsorption column, centrifuge at 12000rpm for 1min, discard the waste liquid, and put the adsorption column into the collection tube.
10. Centrifuge at 12000rpm for 2min and place the adsorption column open at room temperature or 50℃ for several minutes to remove the residual bleach solution in the adsorption column; otherwise, the ethanol in the bleach solution will affect subsequent experiments such as enzyme digestion and PCR.
11. Put the adsorption column into a clean centrifuge tube, add 50-200μL eluent preheated in a water bath at 65℃ to the center of the adsorption film, place at room temperature for 5min, and centrifuge at 12000rpm for 1min.
12. The eluent obtained by centrifugation was added to the adsorption column and centrifuged at 12000rpm for 2min to obtain high quality genomic DNA.

Notes:

1. The sample should avoid repeated freezing and thawing, otherwise the extracted DNA fragments will be small and the extracted amount will decrease.
2. If the solution in the kit is precipitated, it can be re-dissolved in a 65℃ water bath before use, without affecting the extraction effect.
3. If the column is blocked in the centrifugal step of the experiment, the centrifugal time can be appropriately extended.
4. The volume of eluting buffer is better than 50μL, too small volume will affect the recovery efficiency; The pH value of the eluent also has an impact on the elution efficiency. If water is needed to make the eluent, ensure that its pH value is around 8.0 (NaOH can adjust the pH value of water to this range). A pH value lower than 7.0 will reduce the elution efficiency. DNA products should be stored at -20℃ to prevent DNA degradation.
5. DNA concentration and purity detection: The size of the obtained genomic DNA fragments is related to the sample storage time, shear force during operation and other factors. The concentration and purity of the recovered DNA fragments can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer. DNA should have a significant absorption peak at OD260, where an OD260 value of 1.0 corresponds to approximately 50μg/mL double-stranded DNA and 40μg/mL single-stranded DNA. The OD260/0D280 ratio should be 1.7-1.9, if the elution buffer is not used, but deionized water is used, the ratio will be low, because the pH value and the presence of ions will affect the light absorption value, but does not indicate low purity.

Related products:

D1010 6×DNA Loading Buffer
T1060 50×TAE Buffer
T1050 5×TBE Buffer
G8142 GoldView Type II nucleic Acid Stain (5000×)
D1160 Yeast plasmid extraction kit
D1700 Animal tissue/cell genome DNA extraction kit
D1800 Whole blood genome DNA extraction kit

Related literature:

[1] Jian Dong, Guanglu Wang, Cuiying Zhang, et al. A two-step integration method for sea mLess gene deletion in baker's yeast. Analytical Biochemistry. August 2013;30-36. (IF 2.275)

[2] Haigang Tan, Jian Dong, Guanglu Wang, et al. Enhanced freeze tolerance of baker's yeast by overexpressed trehalose-6-phosphate synthase gene (TPS1) and deleted trehalase genes in frozen dough. Journal of Industrial Microbiology & Biotechnology. August 2014. (IF 2.533)

Note: For more information about this product, please refer to Solarbio website.