Fungal Genomic DNA Extraction Kit

Cat No.: D2300

Package: 50T/100T

Storage: Keep it at room temperature, and the reinspection period is one year. (Note: RNase A, proteinase K is shipped as an accessory and stored at-20℃)

Component	50T	100T	Storage
RNase A	100μL	100μL×2	-20℃
Proteinase K	1mL	1mL×2	-20℃
Micro Glass Beads	6g	11g	RT
Solution A	10mL	20mL	RT
Solution B	10mL	20mL	RT
Wash Buffer	15mL	15mL×2	RT
Elution Buffer	10mL	20mL	RT
Adsorption Column	50 units	100 units	RT
Collection Tube	50units	100 units	RT
Instruction	1	1	-

Product description:

Fungi are heterotrophs with eukaryotes and cell walls, with many species, with more than 10,000 reported genera and more than 100000 species. Fungi are usually divided into three categories, namely yeast, mold and fungi (large fungi). This kit is suitable for yeast and mold, can be treated with glass beads, after the early treatment of the bacterial liquid, with silicon membrane adsorption, can get high purity genome. The extracted and purified DNA can be then used directly for downstream applications including PCR/ Real time-PCR, sequencing, Southern blot, mutant analysis, and SNP.

For large fungi, fungi and mushrooms, the extraction effect of this kit will be reduced, so it is not recommended (with recommended treatment methods).

Operation steps (for reference only):

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

1. sample treatment

1) For yeast, 1-2mL of cultured bacterial solution were collected by centrifugation and the supernatant discarded.200 of solution A was added for 2μL RNase A and then 100mg glass beads to shaking on a high-speed oscillator for about 5-10min.

2) Mold (spores can also be treated the same): take 50-100mg of mycelium, add 200μL of solution A, grind the mycelium with A glass grinder, add 2μL RNase A, add 100mg of glass beads, and

shake on the high speed oscillator for about 30min.

3) Large fungus (recommended): weigh 100-200mg sample, pour an appropriate amount of liquid nitrogen, immediately grind it for 3 times, grind the sample into powder, add 400μL of CTAB lysate (Solebo: LS00066), add 2μL RNase A, add 100mg glass beads, oscillate on the high speed oscillator for about 5min, and continue extraction using this kit.

2. 20μL of proteinase K was added, fully mixed, digested in 55℃ water bath for 30min, and the centrifuge tube was mixed several times during digestion. The samples were centrifuged at 12000rpm for 2min. The supernatant was transferred to a new centrifuge tube. If there is precipitation, it can be centrifuged again.
3. Add 200μL of solution B to the supernatant and mix well. If there is white precipitation, 55℃ water bath can be put for 5min, and the precipitation will disappear, without affecting the subsequent experiments. If the solution does not become clear, it means that the sample is not digested thoroughly, which may lead to the amount of extracted DNA less and impure, and may lead to the blocking of the column after the upper column, please increase the digestion time.
4. Then 200μL of anhydrous ethanol is added and fully mixed. At this time, flocculent precipitation may appear, which does not affect the extraction of DNA. The solution and flocculent precipitation are added to the adsorption column for 2min.
5. After centrifugation at 12000rpm for 1min, the waste liquid was discarded and the adsorption column was placed into the collecting tube.
6. Add 600μL of rinse solution to the adsorption column (check whether absolute ethanol has been added before use), centrifuge at 12000rpm for 1min, discard the waste liquid and put the adsorption column into the collecting tube.
7. 600μL of rinse solution was added to the adsorption column, centrifuged at 12000rpm for 1min, the waste solution was discarded and the adsorption column was placed into the collecting tube.
8. It was centrifuged at 12000rpm for 2min to place 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 solution would affect subsequent experiments such as enzyme digestion, PCR, etc.
9. The adsorption column was placed in a clean centrifuge tube, and 50-200μL of eluent preheated with 65℃ water bath was dropped to the center of the adsorption membrane, placed at room temperature for 5min and centrifuged at 12000rpm for 1min.
10. The resulting eluate was added to the adsorption column for 2min at room temperature and at 12000rpm for 2min to obtain high-quality genomic DNA.

Note:

1. Due to the myriad species of fungi, for some particularly difficult to treat fungi, available liquid nitrogen grinding, then with glass beads oscillation, protease K treatment, generally can get a certain amount of genomic DNA, such as electrophoresis detection is very weak, generally PCR will have good results.

2. If precipitated in solution A or B, it can be redissolved in a 55℃ water bath.
3. If the amount of DNA extraction is small, the glass bead processing time can be extended, and if the DNA is extracted into diffuse short bands, the glass bead processing time can be reduced.
4. The volume of the elution buffer should not be less than 50μL, the small volume will affect the recovery efficiency; the pH of the eluate also affects the elution efficiency, the pH value of the water can be NaOH to this range), and the pH value lower than 7.0 will reduce 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 equivalent to approximately 50μg/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 and deionized water is used, the ratio will be low because the pH 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*
M1060	*D2000 DNA Ladder*
M1400	*1kb DNA Ladder*
G8142	*GoldView II nucleic acid stain(5000×)*