ELK Biotechnology
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Plant/Fungal Genomic DNA Extraction Kit

Cat/No.	Specification	Storage/expiration
EP019-50T	50T	Room temperature/1 year
EP019-200T	200T	Room temperature/1 year

Product description

This product is suitable for isolating and purifying total DNA from 100mg~500mg of fresh plant tissue or 20~50 mg of dried plant tissue or 100~300 mg of fungi. After being frozen in liquid nitrogen, the cells and fungal blocks are ground and broken, and the lysate is added to release genomic DNA, and then impurities such as proteins and polysaccharides in plant tissues are extracted and precipitated by chloroform. After the supernatant containing DNA is added to the nucleic acid purification column, the DNA is bound to the nucleic acid purification column, and the residual protein and PCR inhibitors are filtered out, and the DNA is washed with protein-removing solution RPB and Wash Buffer WB, and then washed with Buffer TE. After removal, high-purity DNA can be obtained for various molecular biology experiments.

Kit composition

Components	EP019-50T	EP019-200T	Storage
RNase A	275 μl	1.1 ml	-20 ℃
Buffer PGE	60 ml	240 ml	RT
Buffer RPB	30 ml	120 ml	RT
Wash Buffer WB	60 ml	240 ml	RT
Buffer TE	10 ml	40 ml	RT
Adsorption column P	50 set	200 set	RT
User Manual	1 copy	1 copy	RT

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Reagents and items to be prepared by the user

1. Absolute ethanol, chloroform, isopropanol
2. Liquid Nitrogen and Mortar
3. 2ml centrifuge tube, 1.5ml centrifuge tube and pipette tip
4. Latex gloves, disposable masks and other protective equipment and paper towels
5. Desktop small centrifuge (can be equipped with rotors for centrifuging 1.5ml centrifuge tubes and 2ml centrifuge tubes)
6. Vortex shaker
7. Water bath

Preparation before use

1. If the centrifuge has a cooling function, please set the temperature to 25°C.
2. Set the temperature of the water bath to 65°C, and incubate Buffer PGE and Buffer TE to 65°C.
3. Add absolute ethanol to Buffer RPB and Buffer WB according to the instructions on the label of the reagent bottle, and mark "Alcohol added" in the box on the label.

Operation steps

Plant sample handling:

Put 100-500 mg of plant tissue or 20-50 mg of dried plant tissue (chopped leaves/flowers/stems/roots/seeds are acceptable) into a mortar or homogenizer, add 100-200µl 65℃ preheated Solution PGE and 2 µl β-mercaptoethanol, vigorously grind until homogenous.

- ◆ For tissues with relatively high fiber content, increase the amount of tissue used, and add liquid nitrogen to grind the tissue into powder and then follow step 2, otherwise the recovery rate of DNA will be seriously affected.

Fungal sample processing:

Under the condition of submerging the sample in liquid nitrogen, first grind about 250-300mg of fungal block and bacteria into fine particles, and then quickly grind the bacteria particles into powder after the liquid nitrogen evaporates.

- The fungal cell wall is extremely tough, and liquid nitrogen must be added to grind it into powder to achieve the purpose of fully destroying the cell wall, otherwise the final DNA recovery efficiency will be seriously affected.
- In the process of tissue grinding, liquid nitrogen should be added in time to avoid the melting of ground tissue particles and making it difficult to weigh.
- If the fungal mycelium does not reach 250-300mg (such as a mold colony), scrape off the entire colony, put it in a mortar, add liquid nitrogen, and grind the fungus to a powder.

DNA isolation and extraction:

1. After the grinding is complete, add 800-900 µl 65°C preheated Solution PGE (the total volume of the solution PGE added before is 1ml), and continue grinding for 1 minute to completely lyse the tissue.

2. Transfer 750 µl of the lysate to a user-prepared 2 ml centrifuge tube, and place the centrifuge tube in a 65°C water bath for 30 minutes. Invert the centrifuge tube several times every 5-10 minutes during the water bath to help the release of DNA.

- Prolong the time to 1 hour for tissues with more fibers such as grapes.
- If DNA is extracted from a freshly obtained sample, some RNA in the tissue may be isolated and purified together, but the presence of RNA does not affect PCR-related experiments. To completely remove RNA, add 5 µl RNase A working solution in this step.
- If DNA is extracted from freshly obtained samples, RNA in fungi (especially fungi with high RNA content such as yeast) will usually be isolated and purified together, but the presence of RNA does not affect PCR-related experiments. To completely remove RNA, an additional 5 µl of RNase A can be added at this step.

3. Add 750 µl of chloroform, mix vigorously, and centrifuge at 12000 rpm for 5 minutes.

4. Carefully aspirate the supernatant and transfer to a new 1.5ml tube (the volume is about 600µl at this time).

5. Add an equal volume of isopropanol to the supernatant and mix well.

6. Pipette half the volume of the mixture in step 5 (about 600 µl) into the purification column (the

nucleic acid purification column is placed in a 2ml centrifuge tube), cover the tube cap, and centrifuge at 12,000 rpm for 30 seconds.

7. Discard the filtrate and reinstall the purification column into a 2ml centrifuge tube. Add all the remaining mixture in step 6 to the purification column, cover the tube cap, and centrifuge at 12000 rpm for 30 seconds.

8. Discard the filtrate in the 2ml centrifuge tube, put the nucleic acid purification column back into the 2ml centrifuge tube, add 500 µl Buffer RPB to the nucleic acid purification column, cover the tube cap, and centrifuge at 12000 rpm for 30 seconds.

- The filtrate does not need to be completely discarded. If you want to avoid the contamination of the centrifuge by the filtrate adhering to the nozzle of the centrifuge tube, you can slap the 2ml centrifuge tube upside down on a paper towel once.
- Confirm that absolute ethanol has been added to Buffer RPB.

9. Discard the filtrate in the 2ml centrifuge tube, put the nucleic acid purification column back into the 2ml centrifuge tube, add 600 µl Buffer WB to the nucleic acid purification column, cover the tube cap, and centrifuge at 12000 rpm for 30 seconds.

- Confirm that absolute ethanol has been added to Buffer WB.

10. Discard the filtrate in the 2ml centrifuge tube and repeat step 9.

11. Discard the filtrate in the 2ml centrifuge tube, put the nucleic acid purification column back into the 2ml centrifuge tube, cover the tube cap, and centrifuge at 14000 rpm for 1 minute.

- If the centrifugal speed of the centrifuge cannot reach 14000 rpm, centrifuge at the highest speed for 2 minutes.
- Do not omit this step, otherwise the subsequent experimental results may be affected due to ethanol in the purified nucleic acid.

12. Discard the 2ml centrifuge tube, put the nucleic acid purification column into a clean 1.5ml centrifuge tube, add 100~200 µl 65°C preheated Buffer TE to the purification column, cover the tube cap, and let stand at room temperature for 2 minutes, 12000 rpm Centrifuge for 1 minute.

- If the centrifuge does not have a leak-proof cover, please change the centrifugation condition to 8000 rpm and centrifuge for 1 minute to prevent the tube cover from falling off and

damage the centrifuge.

13. Discard the purification column, and the eluted DNA can be used immediately for various molecular biology experiments; or store the DNA at -20°C for future use.

DNA concentration and purity

DNA concentration (μg/ml) = OD_{260} × 50 × dilution factor, OD_{260}/OD_{280} is about 1.8-2.0

Attention

1. Samples should avoid repeated freezing and thawing, otherwise the extracted DNA fragments will be smaller and the extraction amount will also decrease.
2. All centrifugation steps are performed in a benchtop centrifuge at room temperature.

Frequently Asked Questions and Answers

A. Blocking column:

Suggestion: Please fully lyse the sample and proceed to the next step after there are no obvious flocs; if there are obvious flocs, please carefully absorb the supernatant after centrifugation to avoid clogging the adsorption column.

B. The extraction rate of genome is low:

Suggestion: Increase the amount of samples and Solution PGE in the same proportion to increase the genome yield.

C. There is undissolved precipitate in Buffer

Suggestion: Buffer will precipitate when the temperature is low. Please check whether there is precipitation before use. If there is precipitation, please incubate at 37 ℃ for a while, and use it after the solution is clarified.

D. Ethanol was not added to the Wash Buffer as required

Suggestion: Add the required amount of absolute ethanol according to the instructions, and tighten the bottle cap after use to prevent the ethanol from volatilizing.

E. Selection of dissolution volume and time

Suggestion: The dissolved volume will affect the final yield, the larger the dissolved volume, the

higher the yield, but the concentration will be lower. Please use the dissolution volume recommended by the kit for dissolution to ensure the best yield and concentration. Suggestion: After adding Buffer TE, let it stand at room temperature for 2-5 minutes, which is more conducive to dissolution.