GENCYST

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Plant Genomic DNA Extraction KIT

Catalog No.	Specification	Storage/Shelf life
EP011-50T	50T	Room temperature/1 year
EP011-200T	200T	Room temperature/1 year

Introduction

This kit uses a unique buffer system and is especially suitable for extracting genomic DNA from fresh plant materials. It is safe and convenient to use. It can remove impurities, proteins and other organic compounds in cells to the greatest extent. The extracted genomic DNA fragments are large, high purity, stable and reliable in quality. The DNA recovered by this kit can be used in various routine operations, including enzyme digestion, PCR, library construction, Southern hybridization and so on.

Kit Components

Component	EP011-50T	EP011-200T
Buffer PGE	25 ml	100 ml
Elution Buffer	2.5 ml	10 ml
User Manual	1 copy	1 copy

Before starting

1. If the solution is precipitated, the solution in the kit should be allowed to stand at room temperature for a period of time, if necessary, preheated in a 56 ° C water bath for 10 min to dissolve the precipitate.
2. The sample should be protected from repeated freezing and thawing, otherwise the extracted DNA fragments will be smaller and the amount of extraction will be reduced.
3. Buffer PGE may turn yellow and does not affect the extraction effect.

4. Buffer PGE has precipitation and can be dissolved in 37 C water bath and used after shaking.
5. All centrifugation steps are performed using a benchtop centrifuge and centrifuged at room temperature.
6. Self-prepared chloroform (25:24:1 phenol chloroform isoamyl alcohol may also be required).
7. Chloroform is toxic, please wear protective clothing.

Operation steps

The following steps are for the treatment of 100mg fresh green plant leaves and other tissues, If more tissues are processed, the dosage of different solutions can be scaled up in equal proportions.

1. Material treatment: Take 100mg of fresh tissue of plants or 20mg of dry weight tissue, and fully grind it with liquid nitrogen. Add 600 µl of Buffer PGE and 6 µl of RNaseA (10 mg/ml, with or without addition) and β-mercaptoethanol at a final concentration of 2 mM (with or without addition), vortex for 1 min, and place in a 60 ° C water bath for 20min.

Note: Due to the rich diversity of plant materials, the optimum amount of experimental materials should be selected according to the different materials. For young plant tissues such as Arabidopsis thaliana, pipette tips can be used directly. Broken. Other older organizations recommend using liquid nitrogen grinding.

2. Add 700 µl of chloroform, mix well, and centrifuge at 12,000 rpm (~13,400×g) for 10 min.

Note: If plant tissues rich in polyphenols or starch are extracted, an equal volume extraction can be carried out in Step 2 with phenol:chloroform:isoamyl alcohol=25:24:1.

3. Carefully transfer the upper aqueous phase obtained in the previous step into a new centrifuge tube, add an equal volume of isopropanol, mix well at 12,000 rpm (~13,400 × g) for 10 min, discard the supernatant.

4. Add 75% ethanol to the pellet, mix it upside down, centrifuge at 12,000 rpm (~13,400×g) for 10 min, discard the waste, and repeat this step.

Note: This step is very easy to get rid of the tube wall in ethanol. Care should be taken to remove the supernatant to avoid disturbing the sediment and causing a decrease in recovery.

5. Drain the ethanol carefully with a 10 µl gun and dry the residual ethanol in a clean bench.

6. Add 50-200 µl of Elution Buffer to completely dissolve the DNA product.

DNA concentration and purity Detection

The size of the obtained genomic DNA fragment is related to factors such as sample storage time and shear force during operation. The recovered DNA fragments can be detected for concentration and purity by agarose gel electrophoresis and ultraviolet spectrophotometry. DNA should have a significant absorption peak at OD_{260} with an OD_{260} value of 1 equivalent to approximately 50 µg/ml double-stranded DNA and 40 µg/ml single-stranded DNA. The ratio of OD_{260}/OD_{280} should be 1.7–1.9. If the elution buffer is not used when eluting, and the deionized water is used, the ratio will be lower because the pH value and the presence of ions will affect the light absorption value, but it does not mean the purity is low

Attention

1. The sample should be protected from repeated freezing and thawing, otherwise the extracted DNA fragments will be smaller and the amount of extraction will be reduced.

2. If there is a precipitate in Buffer PGE, re-dissolve in a 56 °C water bath, shake before use.

3. All centrifugation steps were performed using a bench top centrifuge and centrifuged at room temperature.

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Frequently Questions & Answers

No genome was proposed or genome concentration was low.

a, Low yield of genome extraction

Suggestion: Some plant tissues contain few genomes, and the low gelatin concentration is a normal phenomenon. If the follow-up demand is large, it can be extracted and concentrated for many times

b,Selection of volume and time for dissolution

Suggestion: Dissolved volume will affect the final yield, the larger the dissolved volume, the higher the yield, but the concentration will be reduced. Please use the recommended volume of dissolution in the kit to ensure the best yield and concentration. After add Elution Buffer, 2~5 min at room temperature is more favorable for dissolving.