ELK Biotechnology

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DNAsecure Plant Kit

Catalog No.	Specification	Storage/Shelf life
EP010-50T	50T	Room temperature/1 year
EP010-200T	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. This product does not contain harmful chemical components such as phenol, chloroform and so on. It is safer for testers. 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	EP010-50T	EP010-200T	Storage
Buffer PGE	**35 ml**	**140ml**	**RT**
Wash Buffer	**60 ml**	**240 ml**	**RT**
Solution RP	**30 ml**	**120 ml**	**RT**
Elution Buffer	**2.5 ml**	**10 ml**	**RT**
RNase A	**315μl**	**1.25ml**	**-20℃**
Adsorption column	**50 set**	**200 set**	**RT**
User Manual	**1 copy**	**1 copy**	**RT**

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. Customer-supplied: β-mercaptoethanol.

Operation steps

The following steps are for the treatment of 100mg fresh green plant leaves and other tissues

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 Solution 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. 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. Centrifuge at 12,000 rpm (~13,400 xg) for 10 min at high speed and carefully transfer the supernatant to a new centrifuge tube.

3. Optional step (this step is strongly recommended when the solution still contains visible green debris): Centrifuge the supernatant again at 12,000 rpm (~13,400×g) for 5 min and transfer the supernatant to a new centrifuge tube. .

Note: The purpose of this step is to remove the precipitated impurities in the supernatant and make the extracted genomic DNA more pure.

4. Add 0.3 times volume of absolute ethanol to the supernatant, mix well, transfer the supernatant to the adsorption column (if it can not be completely transferred once, it can be divided into words), centrifuge at 10,000 rpm (~10,000×g) 1 min, discard the waste liquid in the collection column.

5. Add 500µl of Solution RP to the adsorption column, put on the collection column, and squeeze it

twice to allow Solution RP to infiltrate into the adsorption membrane to thoroughly wash residual residual proteoglycans. (There may still be green residue after this step, as appropriate Washing once or twice, a small amount of residue does not affect subsequent PCR and other tests). Centrifuge at 10,000 rpm (~10,000 x g) for 1 min and discard the waste from the collection column.

6. Add 600 µl Wash Buffer to the adsorption column, centrifuge at 10,000 rpm (~10,000 × g) for 1 min, and discard the waste liquid from the collection column.

7. Repeat step 6.

8. Centrifuge at 10,000 rpm (~10,000 x g) for 2 min, thoroughly drain the residual ethanol, and discard the waste from the collection column.

9. Take a new EP tube, insert the adsorption column into the tube, open the cap, and let the remaining ethanol dry completely at room temperature for 5-10 minutes.

Note: Residues of ethanol can affect subsequent enzymatic reactions (enzyme digestion, PCR, etc.) experiments.

10. Add 30-50 µl of Elution Buffer to the center of the column to dissolve the precipitate.

Note: Dissolving DNA using the 65°C Elution Buffer can increase the final DNA solution content.

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 OD260 with an OD260 value of 1 equivalent to approximately 50 µg/ml double-stranded DNA and 40 µg/ml single-stranded DNA. The ratio of OD260/OD280 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

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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 Solution 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.

Frequently Questions & Answers

No genome was proposed or genome concentration was low.

a,Column blocked

Use fresh and tender plant samples as much as possible. Repeated cleaning with Solution RP (multiple cleaning results in low genome recovery). Sometimes plugging does not necessarily affect the genome yield, which can be ignored.

b, 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

c， ethanol was not added to Wash Solution as required

Suggestion: Add absolute ethanol according to the instructions, tighten the bottle cap after use to prevent ethanol volatilization.

d,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.