TIANGEN®

DNAsecure Plant Kit

For isolation of genomic DNA from Plants

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DNAsecure Plant Kit

(Spin Column)

Cat. no. 4992707/4992708

Kit Contents

Contents	4992707 50 preps	4992708 200 preps
Buffer LP1	25 ml	100 ml
Buffer LP2	10 ml	40 ml
Buffer LP3	21 ml	84 ml
Buffer PW	15 ml	50 ml
Buffer TE	15 ml	60 ml
RNase A (10 mg/ml)	300 µl	1.25 ml
Spin Columns CB3	50	200
Collection Tubes 2 ml	50	200
Handbook	1	1

Storage

DNAsecure Plant Kit should be stored dry at room temperature (15-30°C) for up to 15 months without showing any reduction in performance and quality. If any precipitate forms in the buffers, it should be dissolved by warming the buffers at 37°C for 10 min before use.

Introduction

DNAsecure Plant Kit provides a fast, simple, and cost-effective genomic DNA miniprep method for routine molecular biology laboratory applications. DNA binds to the silica membrane while contaminants such as proteins are efficiently removed by two wash steps. DNAsecure Plant Kit is ready for use and can be applied to purify Genomic DNA from a variety of plant species and tissues, and the whole process is less than 1 hour. Furthermore, phenol extraction and ethanol precipitation are not required.

Purified DNA is suitable for PCR, restriction digestion, genomic DNA library, and Southern hybridization.

Important Notes Before starting

1. Repeated freezing and thawing of stored samples should be avoided, otherwise decrease in DNA size and lower DNA yield will be generated.
2. Buffer LP1 may become a little yellow upon storage. This does not affect the result.
3. If a precipitate has formed in Buffer LP1 or Buffer LP2, warm buffer at 37°C until the precipitate has fully dissolved.
4. All centrifugation steps should be carried out in a conventional table-top microcentrifuge at room temperature (15-30°C).

Protocol

Ensure that ethanol (96-100%) has been added into Buffer LP3 and Buffer PW as indicated on the tag of the bottle before use.

1. Take 100 mg fresh tissue or 20 mg lyophilized plant tissue and grind them thoroughly in liquid nitrogen. Add 400 µl Buffer LP1 and 6 µl RNase A (10 mg/ml) to the powered plant tissue. Vortex for 1 min to mix. Make sure to disperse all clumps and then incubate for 10 min at room temperature (15-30°C).
2. Add 130 µl Buffer LP2 to the lysate and mix thoroughly by vortex for 1 min.
3. Centrifuge for 5 min at 12,000 rpm (~13,400×g). Pipet the supernatant to a clean 1.5 ml microcentrifuge tube.
4. Add 1.5 volume of Buffer LP3 (For example, add 750 µl Buffer LP3 to 500 µl flow-through) **(Ensure that ethanol (96-100%) has been added into Buffer LP3 before use),** and then immediately mix by vortex for 15 sec. Precipitates may form after the addition of Buffer LP3.

5. Pipet all the mixture from step 4, including any precipitate that may have formed, into the Spin Column CB3 (place the Spin Column CB3 in the Collection Tube). Centrifuge for 30 sec at 12,000 rpm (~13,400×g), and discard the flow-through. Replace the Spin Column CB3 in the Collection Tube.
6. Add 600 µl Buffer PW to the Spin Column CB3 to wash the membrane **(Ensure that ethanol (96-100%) has been added into Buffer PW before use)**, centrifuge for 30 sec at 12,000 rpm (~13,400×g), and discard the flow-through. Replace the Spin Column CB3 back in the Collection Tube.

   Note: If the membrane remains significantly colored (dark, green or yellow), add 500 µl ethanol into the Spin Column CB3. Centrifuge for 30 sec at 12,000 rpm (~13,400×g), and discard the flow-through. Replace the Spin Column CB3 in the Collection Tube.
7. Repeat step 6.
8. Replace the Spin Column CB3 back in the Collection Tube, centrifuge for 2 min at 12,000 rpm (~13,400×g) to remove residual Buffer PW. Open the lid of the Spin Column CB3 and incubate the assembly at room temperature for several minutes to dry membrane completely.

   Note: Residual ethanol from Buffer PW inhibits subsequent enzymatic reactions (e.g. restriction enzyme digestion and PCR).
9. Discard the Collection Tube and transfer the Spin Column CB3 to a clean 1.5 ml microcentrifuge tube. Pipet 50-200 µl Buffer TE directly onto the Spin Column CB3 membrane, incubate for 2-5 min at room temperature and then centrifuge for 2 min at 12,000 rpm (~13,400×g) to elute DNA.

   Note: For increased DNA concentration, add the solution obtained from step 9 to the center of membrane again. Incubate at room temperature for 2 min, and then centrifuge for 2 min at 12,000 rpm (~13,400×g). If the volume of Buffer TE is less than 50 µl, it may affect recovery efficiency. What's more, the pH value of elution buffer has some influence in eluting; we suggest choosing buffer TE or distilled water (pH7.0-8.5) to elute gDNA. For long-term storage of DNA, eluting in Buffer TE and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.