TIANGEN®

Plant Genomic DNA Kit

For purification of genomic DNA from plant cells

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Plant Genomic DNA Kit

(Spin Column)

Cat. no. GDP305

Kit Contents

Contents	GDP305-02 50 preps	GDP305-03 200 preps
Buffer GP1	40 ml	160 ml
Buffer GP2	40 ml	160 ml
Buffer GD	13 ml	52 ml
Buffer PW	15 ml	50 ml
Buffer TE	15 ml	60 ml
Spin Columns CB3	50	200
Collection Tubes 2 ml	50	200
Handbook	1	1

Storage

Plant Genomic DNA Kit could 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

The Plant Genomic DNA Kit provides a fast, simple, and cost-effective genomic DNA extraction method for routine molecular biology laboratory applications. The Kit uses silica membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. The kit is ready for use and can purify the genomic DNA from a wide variety of plant species and tissues, and the whole process is completed in less than 1 hour.

Purified DNA is suitable for PCR, restriction endonuclease digestion and Southern Hybridization.

Yield

Sample	Wet Weight	DNA Yield
Plant tissues	100 mg	3-30 μg

Note: DNA yields vary with different plant samples.

Important Notes

1. Repeated freezing and thawing should be avoided; otherwise it would reduce the DNA size and quantity.
2. If a precipitate formed in Buffer GP1 or Buffer GP2, warm buffer to 37°C until the precipitate has fully dissolved.

Protocol

Ensure that Ethanol (96-100%) has been added into Buffer GD and Buffer PW before use.

1. Place 100 mg wet weight plant tissue or 30 mg lyophilized plant tissue and grind the samples thoroughly in liquid nitrogen.
2. Add 700 μl 65°C pre-heated GP1 (β-Mercaptoethanol (β-ME) should be added to Buffer GP1 before use. The final concentration of β-ME is 0.1%) to the powdered plant tissue, Vortex for 10-20 sec to mix, make sure to disperse all clumps and then Incubate for 20 min at 65°C, mix by inverting the tube for several times.
3. Add 700 μl chloroform, mix by inverting the tube for several times, centrifuge for 5 min at 12,000 rpm (~13,400 × g).

   Note: If the plant sample contains polyphenol or starch, 1:1 of Phenol/ Chloroform could be used before step 3 to extract plant genomic DNA.

4. Pipet the supernatant water phase to a new tube, add 700 µl Buffer GP2, mix by inverting the tube for several times.
5. Pipet all of the mixture from step 4 into the Spin Column CB3 (place the Spin Column CB3 in the Collection Tube first). Close the CB3 lid and centrifuge for 30 s at 12,000 rpm (~13,400 × g). Discard the filtrate and place the Spin Column CB3 into the Collection Tube.

   If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same column.
6. Add 500 µl Buffer GD **(Ensure that ethanol (96-100%) is added to Buffer GD before use)**, centrifuge at 12000 rpm (~13,400 × g) for 30 s then discard the filtrate and place the Spin Column CB3 back into the Collection Tube.
7. Add 600 µl PW **(Ensure that ethanol (96-100%) is added to Buffer PW before use)** to the Spin Column CB3, and centrifuge for 30 s at 12,000 rpm (~13,400 × g), discard the flow-through, place the Spin Column CB3 back into the Collection Tube.
8. Repeat step 7.
9. Place the Spin Column CB3 in the Collection Tube, centrifuge for 2 min at 12,000 rpm (~13,400 × g), discard the flow-through. Open lid of CB3 and stay at room temperature for a while to dry the membrane completely.

   Note: Residual ethanol from Buffer PW may inhibit subsequent enzymatic reactions.
10. Place Spin Column CB3 to a new centrifuge tube, and add 50-200 µl Buffer TE directly onto the CB3 membrane, incubate for 2-5 min at room temperature (15-30°C), and then centrifuge for 2 min at 12,000 rpm (~13,400 × g) to elute.

    Note: Elution with small volumes (<50 µl) will reduce the efficiency of the elution and the DNA yield. The pH value of elution buffer will have some influence in eluting; Buffer TE or distilled water (pH 7.0-8.5) is suggested to elute DNA. 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. To improve the yield of genomic DNA obtained, the elution buffer could be added to Spin Column CB3 again, stand for 2 min at room temperature (15-30°C), then centrifuge at 12,000 rpm (~13,400 × g) for 2 min to elute.