TIANGEN®

N96 Plant Genomic DNA Kit

For high -throughput purification of total cellular DNA from plant cells

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N96 Plant Genomic DNA Kit

(Spin Plate)

Cat. no. 4992719

Kit Contents

Contents	4992719 (2 plates)
Buffer GP1	160 ml
Buffer GP2	160 ml
Buffer GD	52 ml
Buffer PW	50 ml
Buffer TE	60 ml
N96 Plate CB3 (H)	2
N96 Well Plate	6
Plate Cover	12
Handbook	1

Storage

The Kit could be stored dry at room temperature (15-25°C) for up to 12 months. For long term storage, this kit should be stored at 2-8°C. If a precipitate has formed in Buffer under 2-8°C, please place the buffer at room temperature or warm at 37°C for 10 min to dissolve the precipitate.

Introduction

N96 Plant Genomic DNA Kit adopts spin plate which can specifically bind DNA and unique buffer system for plant gDNA extraction. The silica membrane within the spin plate is made of TIANGEN's exclusive material which would specifically bind DNA. Proteins and other impurities can be properly removed, and genomic DNA extracted by this kit is integrate, pure and stable.

The genomic DNA extracted by this kit is suitable for lots of downstream experiments which include restriction enzyme digestion, PCR analysis, library construction, Southern blot, etc.

Extraction Yield

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

Note: DNA yields vary with different plant samples.

Product Features

Simple and fast: Genomic DNA can be easily extracted within 1 h.

High purity: Extracted gDNA can be directly used in biological experiments like PCR, enzyme digestion and southern blot.

Important Notes Before Starting

1. Avoid repeated freezing and thawing of samples, otherwise the DNA integrity and yield will be reduced.
2. If any precipitate has been formed in Buffer GP1 or Buffer GP2, warm the buffer at 37°C until the precipitate has fully dissolved.
3. All centrifugation steps should be carried out in a conventional table-top microcentrifuge at room temperature (15-25°C).

Protocol

Ensure that ethanolhas been added into Buffer GD and Buffer PW as indicated on bottle tag before use.

1. Take 100 mg wet weight plant tissue or 30 mg lyophilized plant tissue and grind the samples thoroughly in liquid nitrogen.
2. Move the powdered plant tissue into a microcentrifuge tube which has 700 µl 65°C pre-heated Buffer GP1 **(β-Mercaptoethanol (β-ME) should be added to Buffer GP1 before use. The final concentration of β-ME is 0.1%)** in it, mix by quick inverting, then incubate for 20 min in a 65°C water bath. Mix by inverting the tube for several times during the incubation.
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: For polyphenol or starch-rich plant samples, 1:1 of phenol/ chloroform could be used before step 3 to extract plant genomic DNA.
4. Carefully pipet the supernatant water phase to a N96 Well Plate, add 700 µl Buffer GP2, mix thoroughly.
5. Pipet all of the mixture from step 4 into a N96 Plate CB3 (place the N96 Plate CB3 in a N96 Well Plate first). Centrifuge for 5 min at 3,600 rpm (~2,130 × g). Discard the flow-through and place the N96 Plate CB3 back into the N96 Well Plate.
6. Add 600 µl Buffer GD **(Ensure that ethanol is added before use)** to the N96 Plate CB3, centrifuge at 3,600 rpm (~2,130 × g) for 5 min, discard the flow-through and place the N96 Plate CB3 back into the N96 Well Plate.
7. Add 600 µl Buffer PW **(Ensure that ethanol is added before use)** to the N96 Plate CB3, and centrifuge for 5 min at 3,600 rpm (~2,130 × g), discard the flow-through, place the N96 Plate CB3 back into the N96 Well Plate.
8. Add 600 µl Buffer PW to the N96 Plate CB3, and centrifuge for 5 min at 3,600 rpm (~2,130 × g), discard the flow-through.

   Note: If the plate membrane shows green, please add 500 µl ethanol to the N96 Plate CB3, centrifuge for 5 min at 3,600 rpm (~2,130 × g), discard the flow-through, and place the N96 Plate CB3 back into the N96 Well Plate.

9. Place the N96 Plate CB3 back into the N96 Well Plate, centrifuge for 5 min at 3,600 rpm (~2,130 × g), discard the flow-through. Keep the N96 Plat e CB3 at room temperature for several minutes to dry the membrane completely.

   Note: The purpose of this step is to completely remove washing buffer from the N96 Plate CB3. Residual ethanol from Buffer PW may have negative effect on subsequent enzymatic reactions (like PCR and enzyme digestion).

10. Place N96 Plate CB3 in a new N96 Well Plate, and add 50-200 µl Buffer TE directly onto the center of CB3 membrane, incubate for 2-5 min at room temperature (15-25°C), and then centrifuge for 8 min at 3,600 rpm (~2,130 × g) to elute DNA.

    Note: The volume of Buffer TE should not be less than 50 µl, or else the yield would be affected. The pH value of elution buffer has significant influence on eluting; if distilled water is used to elute DNA, the pH value should be within 7.0-8.5. Low pH value (pH<7) would reduce the efficiency of eluting. Extracted genomic DNA should be stored at -20°C to avoid degradation. To improve the yield of genomic DNA, eluate could be pipetted back to N96 Plate CB3 again, stand for 2 min at room temperature (15-25°C), then centrifuge at 3,600 rpm (~2,130 × g) for 5-8 min to elute.

Determination of DNA Concentration and Purity

The size of the obtained genomic DNA fragment is related to factors such as sample storage time and shearing force during operation. The obtained DNA fragments can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer for concentration and purity.

DNA should have a significant absorption peak at OD_{260}, with OD_{260} value of 1 equivalent to about 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 using deionized water instead of elution buffer, the ratio will be low, because the pH value and the presence of ions will affect the light absorption value, but it does not mean the purity is low.