Ver.25021

ODP305

Plant Genomic DNA Kit

For extraction of DNA from plant tissues

ISO 13485:2016 ISO 9001:2015

Plant Genomic DNA Kit

(ODP305)

Kit Contents

Contents	50 Preps
Buffer GP1	40mL
Buffer GD	13mL
Buffer AB	5mL
Buffer PW	15mL
Buffer TE	10mL
Spin Column CB3 with Collection Tubes	50

Storage

Plant DNA Kit should be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer storage, the kit can be stored at 2-8°C.

(Note: Check buffers for precipitate before use and dissolve at 37°C for 10 minutes if necessary.)

Introduction

The Plant 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 extract the genomic DNA from a wide variety of plant species and tissues, and the whole process is completed in less than 1 hour. Extracted DNA is suitable for PCR, restriction endonuclease digestion and Southern blotting.

DNA Yield: 3-30µg genomic DNA from 100mg plant tissue.
Note: Yield may vary relative to the type of sample, genomic size, ploidy, age of the sample etc. Young leaves or needles will give best result.

Chemicals required but not provided

1. Chloroform
2. β-Mercaptoethanol
3. RNase A (Cat# ORT405)
4. Ethanol (96-100%)

Important Notes

1. Add appropriate amount of ethanol (96-100%) to Buffer GD and Buffer PW as indicated on the bottle before use.
   Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.
2. All centrifugation steps are carried out at 13,000 ×g (12,000 rpm) in conventional tabletop microcentrifuge at room temperature.
3. Increasing the time of absorption and elution could improve recovery efficiency for 10kb DNA fragment.
4. The recovery efficiency is related to starting DNA quantity and elution volume. The less starting quantity or elution volume, less the recovery efficiency.

Protocol

Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.

1. Place 100mg fresh sample or frozen sample in a 1.5mL microcentrifuge tube.
   Note: Samples may be frozen by dipping them in liquid nitrogen. Samples should be finely chopped before transferring to the microcentrifuge tube.
2. Add 700µL Buffer GP1 to the sample in 1.5mL microcentrifuge tube.
3. Add 14µL β-Mercaptoethanol each tube. Vortex for 10 to 20 seconds to mix, make sure to disperse all clumps and then incubate for 20 minutes at 65°C, mix by inverting the tube for several times during incubation.
   Note: Treat this mixture with RNase A to remove RNA. Add 5µL RNase A, mix by inverting the tube for several times and keep the tube for 5 minutes incubation at room temperature.
4. Add 700µL chloroform, mix by inverting the tube for several times. Centrifuge for 5 minutes at 12,000 rpm (~13,400 ×g).
5. Pipette the supernatant to a new tube, add 800µL ethanol, and mix by inverting the tube several times.
   Before transferring the lysate to spin column CB3, add 100µL Buffer AB to the center of spin column CB3 (in a 2mL collection tube), incubate at room temperature for 5 minutes. Centrifuge at 12,000 rpm for 2 minutes and discard flow-through.
6. Pipette all of the mixtures from step 5, including any precipitate that may have formed, into the spin column (place the spin column in the collection tube). Close the lid and incubate for 10 minutes. Centrifuge for 30 seconds at 12,000 rpm (~13,400 ×g). Discard the filtrate and set the spin column into the collection tube.

Note: Since the capacity of spin column CB3 is 700µL, the centrifugation step must be repeated for processing all the mixture from step 5.

7. Carefully open the spin column and add 500µL Buffer GD (Ensure that ethanol (96-100%) is added to Buffer GD before use), Close the lid and centrifuge at 12000 rpm (~13,400 × g) for 30 seconds then discard the filtrate and place the spin column into the collection tube.
   Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.
8. Add 700µL Buffer PW (Ensure that ethanol is added to Buffer PW before use) to the spin column to wash the membrane, and centrifuge for 30 seconds at 12,000 rpm (~13,400 ×g), discard the flow-through, replace the spin column in the collection tube.
9. Repeat step 8.
10. Replace the spin column in the collection tube, centrifuge for 2 minutes at 12,000 rpm (~13,400 ×g) to remove residual wash Buffer PW. Discard the collection tube and transfer the spin column CB3 to a clean 1.5mL or 2mL microcentrifuge tube. Open the lid of the spin column CB3 and incubate the assembly at room temperature (15-25°C) for 2 minutes to dry membrane completely.
    Note: Residual ethanol from Buffer PW may inhibit subsequent enzymatic reactions.
11. Pipette 100µL Buffer TE directly onto the membrane, incubate for 2-5 minutes at room temperature (15–25°C), and then centrifuge for 2 minutes at 12,000 rpm (~13,400 ×g) to elute.
    Note: To increase the DNA yield, introduce the eluted Buffer TE to the column and centrifuge for 2 minutes at 12,000 rpm. The volume of Buffer TE must be more than 30µL, or it may affect recovery efficiency. What's more, the pH value of eluted buffer will have some influence in elution; we suggest Buffer TE or distilled water (pH 7.0-8.5) 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.