Ver. 23121

ODP306

Plant Genomic DNA Kit – Premium

For extraction of genomic DNA from mucilaginous plants

ISO 13485:2016 ISO 9001:2015

Plant Genomic DNA Kit – Premium

Spin Column
(ODP306)

Kit Contents

Contents	50 Preps
Buffer MET	100mL
Buffer GP2	40mL
Buffer GD	13mL
Buffer PW	15mL
Buffer TE	10mL
Spin column CB3	50
Collection Tubes	50
Handbook	1

Storage

Plant Genomic DNA Kit - Premium can 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 Genomic DNA Kit – Premium 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 mucilaginous plant tissues. The whole process is completed in less than 1 hour. Extracted DNA is suitable for PCR, restriction endonuclease digestion and Southern hybridization.

Chemicals required but not provided

1. Isopropanol
2. Chloroform
3. β-Mercaptoethanol
4. RNaseA (Cat#ORT405)
5. 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.
2. All centrifugation steps are carried out at 13,000 ×g (12,000 rpm) in conventional tabletop centrifuge 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 or frozen sample in a 1.5mL microcentrifuge tube.
   Note: Samples should be finely chopped before transferring to the microcentrifuge tube.
2. Add 1mL of Buffer MET to the tube containing the sample.
3. Add 10μL β-Mercaptoethanol to the tube.
4. Vortex for 30 seconds and centrifuge at 5,000 rpm for 5 minutes.
5. Discard supernatant.
   Note: The Buffer MET wash may be repeated for challenging samples where the supernatant from the first wash is found to be viscous, densely turbid or dark brown in color.
6. Add 700μL Buffer GP2 to the pellet.
7. Add 14μL β-Mercaptoethanol to tube.
8. Vortex for 30 seconds, 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 RNaseA (Cat#ORT405, not supplied) to remove RNA. Add 5μL RNaseA, mix by inverting the tube for several times and keep the tube for 5 minutes incubation at room temperature.
9. Add 700μL Chloroform, mix by inverting the tube for several times, centrifuge for 5 minutes at 12,000 rpm (~13,400 ×g).
10. Pipette the supernatant to a new tube, add 700μL ice-cold Isopropanol, and mix by inverting the tube several times.
    Note: The supernatant may still be slightly viscous. This does not affect the subsequent steps.
11. Pipette all of the mixtures from step 10, including any precipitate that may have formed, into the spin column CB3 (place the spin column CB3 in the collection tube). Close the lid and incubate for 10 minutes at room temperature. Centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds. Discard the filtrate and replace the spin column CB3 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 10.

12. Carefully open the spin column CB3 and add 500µL Buffer GD. Close the lid and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds then discard the filtrate and replace the spin column CB3 into the collection tube.
13. Add 700µL Buffer PW to the spin column CB3 to wash the membrane, and centrifuge for 30 seconds at 12,000 rpm (~13,400 ×g), discard the flow-through, and replace the spin column CB3 in the collection tube.
14. Repeat step 13.
15. 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.
16. Pipette 50–200µL 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: The volume of Buffer TE must be at least 50µL, or it may affect recovery efficiency. What's more, the pH value of eluted buffer will have some influence in eluting, 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.

Notes:

- The initial amount of sample can be increased if the yield is lower than required. Increase the amount of Buffer MET, Buffer GP2, β-Mercaptoethanol, chloroform and isopropanol accordingly.