

ODP306

# Plant Genomic DNA Kit – Premium

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For extraction of genomic DNA from mucilaginous plants



# Plant Genomic DNA Kit – Premium

Spin Column  
(ODP306)

## Kit Contents

Contents	50 Preps
Buffer MET	100mL
Buffer GP3	40mL
Buffer SA	5mL
Buffer AB	5mL
Buffer GD	13mL
Buffer PW	15mL
Buffer TE	10mL
Spin Columns (CB3) & Collection Tubes	50 Nos

## 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. Extracted DNA is suitable for PCR, restriction endonuclease digestion and Southern hybridization.

## Chemicals required but not provided

1. Isopropanol
2. Chloroform
3. Isoamyl alcohol
4.  $\beta$ -Mercaptoethanol
5. RNase A (Cat#ORT405)
6. 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. Increasing the time of absorption and elution could improve recovery efficiency for 10kb DNA fragment.
3. All centrifugation steps are carried out at 13,000 rpm in conventional tabletop microcentrifuge at room temperature.
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.**

**Prepare an aliquot of Buffer GP3 & TE and pre-warm at 65°C for 2 minutes before step 6 & 17.**

1. Place 100mg fresh 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 1mL of Buffer MET to the tube containing the sample.
3. Add 10 $\mu$ L  $\beta$ -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 700 $\mu$ L pre-warmed Buffer GP3 to the pellet.
7. Add 14 $\mu$ L  $\beta$ -Mercaptoethanol to tube.
8. Vortex for 30 seconds, make sure to disperse all clumps and then incubate for 30-45 minutes at 65°C, mix by inverting the tube for several times during incubation.

**Note: For some samples, incubation may be extended up to 60 minutes depending on the sample type.**

**Treat this mixture with RNase A (Cat#ORT405, not supplied) to remove RNA. Add 5 $\mu$ L RNase A, mix by inverting the tube for several times and keep the tube for 5 minutes incubation at room temperature.**

9. Add 600 $\mu$ L Chloroform:isoamyl alcohol (24:1), mix by vortexing the tube, centrifuge for 15 minutes at 12,000 rpm (~13,400  $\times$ g).

**Note: Chloroform:isoamyl alcohol (24:1) has to be prepared freshly before extraction.**

10. Carefully transfer the aqueous phase to a fresh tube.

**Note:** For samples with high polysaccharides content, repeat the chloroform:isoamyl alcohol extraction for both the aqueous and organic phases, then combine the aqueous phases from both tubes into a new tube and continue the extraction.

11. Add 100 $\mu$ L Buffer SA followed by equal volume of ice-cold isopropanol (Aqueous phase:isopropanol = 1:1). Mix by gentle inversion.

**Note:** The supernatant may still be slightly viscous. This does not affect the subsequent steps.

12. Incubate at -20°C for 1 hour to enhance DNA precipitation.

**Note:** The extraction can be paused at this stage and continued on the next day. The mixture needs to be stored at -20°C during this pause point.

**Before transferring the lysate to spin column CB3, add 100 $\mu$ L Buffer AB to the center of spin column CB3 (in a 2mL collection tube), and incubate at room temperature for 5 minutes. Centrifuge at 12,000 rpm for 2 minutes and discard flow-through.**

13. Pipette all the mixtures from step 12, 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  $\times$ g) for 30 seconds. Discard the filtrate and replace the spin column CB3 in to the collection tube.

**Since the capacity of spin column CB3 is 700 $\mu$ L, the centrifugation step must be repeated for processing all the mixture from step 12.**

14. Carefully open the spin column CB3 and add 500 $\mu$ L Buffer GD. Close the lid and centrifuge at 12,000 rpm (~13,400  $\times$ g) for 30 seconds then discard the filtrate and replace the spin column CB3 into the collection tube.
15. Add 700 $\mu$ L Buffer PW to the spin column CB3 to wash the membrane, and centrifuge for 30 seconds at 12,000 rpm (~13,400  $\times$ g), discard the flow-through, and replace the spin column CB3 in the collection tube.
16. Centrifuge for 2 minutes at 12,000 rpm (~13,400  $\times$ 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.

17. Pipette 50–100 $\mu$ L Buffer pre-warmed 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  $\times$ 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.

To increase DNA yield, introduce the eluted buffer TE to the spin column CB3 and centrifuge for 2 minutes at 12,000 rpm.