

ODP307

# Plant Genomic DNA Kit – Pro

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For extraction of total cellular DNA from plant tissues  
with high secondary metabolites



# Plant Genomic DNA Kit – Pro

Spin Column  
(ODP307)

## Kit Contents

| Contents         | 50 Preps |
|------------------|----------|
| Buffer GP2       | 40mL     |
| Buffer AB        | 5mL      |
| Buffer GD        | 13mL     |
| Buffer PW        | 15mL     |
| Buffer TE        | 10mL     |
| Spin Column CB3  | 50       |
| Collection Tubes | 50       |
| Handbook         | 1        |

## Storage

Plant Genomic DNA Kit - Pro can be stored dry at room temperature (15-25°C) for up to 24 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

Plant Genomic DNA Kit - Pro 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 tissues with high secondary metabolite contents. The whole process is completed in 1 hour. Extracted DNA is suitable for PCR, restriction endonuclease digestion and Southern hybridization.

**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)
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

**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.
- Add 100µL Buffer AB to the center of spin column, keep at room temperature for 5 minutes and then centrifuge at 12000 rpm for 2 minutes, before using the spin column.

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 GP2 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.

**Optional step: Treat this mixture with RNase A (Cat# ORT405, not supplied) 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 450µL ethanol (96-100%), and mix by inverting the tube several times.

**Note: Before transferring the lysate to spin column, add 100µL Buffer AB to the center of spin column, keep at room temperature for 5 minutes and then centrifuge at 12000 rpm for 2 minutes.**

6. Pipette all of the mixtures from step 5, including any precipitate that may have formed, into the spin column CB3 and close the lid (place the spin column CB3 in the collection tube). Incubate at room temperature for 10 minutes. Centrifuge for 30 seconds at 12,000 rpm ( $\sim 13,400 \times g$ ). Discard the filtrate and set the spin column CB3 into the collection tube.

**Note: Since the capacity of spin column CB3 is 700 $\mu$ L, the loading-centrifugation step must be repeated for processing all the mixture from step 5.**

7. Carefully open the spin column CB3 and add 500 $\mu$ L Buffer GD (Ensure that ethanol is added to Buffer GD before use). Close the lid and centrifuge at 12,000 rpm ( $\sim 13,400 \times g$ ) 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 700 $\mu$ L Buffer PW (Ensure that ethanol is added to Buffer PW before use) to the spin column CB3 to wash the membrane, and centrifuge for 30 seconds at 12,000 rpm ( $\sim 13,400 \times g$ ), discard the flow-through, replace the spin column CB3 in the collection tube.

9. Repeat step 8.

10. Replace the spin column CB3 in the collection tube, centrifuge for 2 minutes at 12,000 rpm ( $\sim 13,400 \times g$ ) 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) 2 minutes to dry membrane completely.

**Note: Residual ethanol from Buffer PW may inhibit subsequent enzymatic reactions.**

11. Pipette 50–200 $\mu$ L 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 ( $\sim 13,400 \times g$ ) to elute.

**Note: The volume of Buffer TE must be more than 30 $\mu$ L, 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 GP2,  $\beta$ -Mercaptoethanol, chloroform and isopropanol accordingly.