

10. Put the adsorption column into a new centrifuge tube (self-provided), add 50-100  $\mu$ L Buffer GE or RNase-Free water to the middle of the adsorption membrane, leave it at room temperature for 2-5 min, 12,000 rpm centrifuge for 1 min,  $-20^{\circ}\text{C}$  to preserve the DNA.

**Note:** 1) If the downstream experiment is sensitive to pH or EDTA, you can use sterilized water for elution. The pH of the eluent has a great influence on the elution efficiency. If water is used as the eluent it should be ensured that its pH is 7.0-8.5 (the pH of water can be adjusted to this range with NaOH), and the elution efficiency is not high when the pH is lower than 7.0.

2) Incubation at room temperature for 5 min before centrifugation can increase the yield.

3) If the final concentration of DNA is to be increased, the DNA eluate obtained in step 10 can be re-spiked onto the adsorbent membrane and step 10 repeated; if the volume of elution is less than 100  $\mu$ L, the final concentration of DNA can be increased, but it may reduce the total yield of DNA. If the amount of DNA obtained is less than 1  $\mu$ g, it is recommended to use 50  $\mu$ L Buffer GE for elution.

4) Because DNA stored in water will be affected by acidic hydrolysis, for long-term storage, it is recommended that the DNA be eluted with Buffer GE and stored at  $-20^{\circ}\text{C}$ .

## NuClean Plant Genomic DNA Kit

**Cat. No. :** CW0531S (50 preps)

CW0531M (200 preps)

**Storage Condition:** Store at room temperature ( $15-30^{\circ}\text{C}$ ).

### Components

Component	CW0531S (50 preps)	CW0531M (200 preps)
Buffer LP1	25 mL	100 mL
Buffer LP2	10 mL	40 mL
Buffer LP3 (concentrate)	21 mL	84 mL
Buffer GW2 (concentrate)	15 mL	75 mL
Buffer GE	15 mL	60 mL
RNase A (10 mg/mL)	300 $\mu$ L	1.25 mL
Spin Columns DM with Collection Tubes	50	200

## Introduction

This kit uses centrifugal adsorption columns with high efficiency and specific binding of nucleic acids and a unique buffer system, which is suitable for extracting genomic DNA from a wide variety of different fresh or frozen plant tissues with maximum removal of impurities from the plant tissues. The kit does not need to be extracted by phenol / chloroform and is safe to operate. The extracted genomic DNA fragments are large, pure, stable and reliable in quality, and are suitable for PCR, fluorescent quantitative PCR, molecular markers, library construction and other experiments.

## Reagents to be Supplied by user

100% ethanol

## Precautions

1. Repeated freezing and thawing of the samples should be avoided, as this may result in smaller fragments of extracted DNA and a decrease in the amount extracted.
2. 100% ethanol should be added to Buffer LP3 and Buffer GW2 according to the instructions on the label of the reagent bottle before first use.
3. Please check Buffer LP1 and Buffer LP2 for crystallization or precipitation before use. If there is any crystallization or precipitation, please re-dissolve Buffer LP1 and Buffer LP2 in a 56°C water bath.

## Protocol

1. Take about 100 mg of fresh plant tissue or about 20 mg of dry weight tissue and add liquid nitrogen to grind it fully.
2. Collect the ground powder into a centrifuge tube (self-provided), add 400  $\mu$ L Buffer LP1 and 6  $\mu$ L RNase A (10 mg/mL), vortex for 1 min, and leave at room temperature for 10 min to allow sufficient cleavage.

**Note:** 1) Use vortex shaking or pipette blowing to fully lyse the tissue, incomplete tissue lysis will affect the final DNA yield.

2) Do not mix Buffer LP1 with RNaseA before use.

3. Add 130  $\mu$ L of Buffer LP2, mix well, and vortex for 1 min.
4. Centrifuge at 12,000 rpm ( $\sim$ 13,400  $\times$ g) for 5 min, and transfer the supernatant to a new centrifuge tube (self-provided).
5. Add 1.5 times the volume of Buffer LP3 (please check whether 100% ethanol has been added before use) and mix well (e.g., 500  $\mu$ L of filtrate to 750  $\mu$ L of Buffer LP3).

**Note:** Mix immediately after adding Buffer LP3, precipitation may occur but will not affect subsequent experiments.

6. Add all of the solution and precipitate obtained in the previous step to the Spin Columns DM that have been loaded into the collection tube, if the solution cannot be added all at once, it can be transferred in several times. Centrifuge the column at 12,000 rpm for 1 min, discard the waste liquid and put the column back into the collection tube.
7. Add 500  $\mu$ L of Buffer GW2 to the column (please check whether 100% ethanol has been added before use), centrifuge at 12,000 rpm for 1 min, discard the waste solution, and return the column to the collection tube.

**Note:** If the adsorbent membrane appears green, add 500  $\mu$ L of 100% ethanol to the adsorbent column, centrifuge at 12,000 rpm for 1 min, discard the waste solution, and put the adsorbent column back into the collection tube.

8. Repeat step 7.
9. Centrifuge at 12,000 rpm for 2 min and pour off the waste solution in the collection tube. Leave the adsorbent column at room temperature for several minutes to dry thoroughly.

**Note:** The purpose of this step is to remove the residual ethanol from the adsorbent column; ethanol residue can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).