# Plant Genomic DNA Miniprep Kit

For research use only

## Kit Content

| Content | DN1342 |
|---|---|
| RNase A | 150 µl |
| Solution YI | 15 ml |
| Solution YII | 15 ml |
| Solution YIII | 20 ml |
| Wash buffer DPB | 30 ml |
| Wash buffer DW | 30 ml |
| Eluent buffer | 5 ml |
| Spin column | 50 each |

## Description

**Plant Genomic DNA Miniprep Kit is designed for rapid and cost-effective small-scale preparation of high quality Plant Genomic DNA. The method is based on silicamembrane spin column.This method requires few manipulations, and is both faster and easier to perform than other organic-based extraction methods. The purified DNA is suitable for all common molecular biology procedures, including restriction digestion, cloning, sequencing, etc.**

## Applications

**Purified DNA is free from contaminants and enzyme inhibitors, and typically has A260/A280 ratios between 1.7 and 1.9, and is suitable for applications such as:**

- **Transformationn**
- **PCR**
- **DNA Sequencing**
- **in vitro transcription**

## Feature.

- **High purity : A260/A280=1.7-1.9. no chloroform and phenol, which is safe for people.**

## Store

**Store RNase A at -20$^{0}$C, other reagents can be store at room temperature for up to 1 year. Any precipitate forms in the buffers during storage; it should be redissolved by incubating the buffers at 37°C before use. After addition of RNase A, the Resuspension Solution can be used for 6 months when stored at 4°C.**

## Note

- **Add the provided RNase A solution to the Solution YI and mix.**
- **Prior to the initial use of the kit, dilute the Wash Buffer W with ethanol (96-100%):**

| | DN1342 |
|---|---|
| Wash Buffer(DW) | 30 ml |
| Ethanol | 45 ml |
| Total Volume | 75 ml |

**After the ethanol has been added, mark the check box on the bottle to indicate the completed step.**

• Solution YI, Solution YII, and Wash buffer DPB contain irritants. Wear gloves when handling these solutions.
• All purification steps should be carried out at room temperature.
• All centrifugations should be carried out in a table-top microcentrifuge at>12000 g (10,000-14,000 rpm, depending on the rotor type)

## Protocol

1. Transfer 1~5 ml overnight culture (≤5 x 107 cells) to a sterile microcentrifuge tube.Centrifuge at room temperature for 1 min at ~12,000 rpm. Discard the supernatant.
2. Resuspend the cell pellet in 300 µl lyticase buffer and 50 U lyticase by pipetting up and down gently several times or by vortexing until the mixture is homogeneous. Incubate at room temperature for 30 minutes.

**Note** ： Lyticase (Cat. # N9031/N9032) can be purchase according to your needs. The length of time required may vary for different species.

3. Centrifuge at room temperature for 10 minutes at ~5,000 rpm. Discard the supernatant.
4. Add 250 µl Solution Y I (mixed with RNase A), resuspend the pellet by vortexing until the mixture is homogeneous. Incubate at room temperature for 1~2 minutes.

**Note.** • Ensure RNase A has been added to the Solution Y I.
• The culture should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

5. Add 250 µl Solution Y II. Mix gently by inverting the capped tube 5-6 times. Do not vortex. Incubate at room temperature for a maximum of 5 minutes.

**Note:** • Vortexing will contaminate the suspension with plant chromosomal DNA.
• Incubate until solution clears. Over-incubation at this stage may reduce the quality of your purified plasmid DNA.

6. Add 350 µl Solution Y III and invert the capped tube 5-6 times or until a white, free-flowing precipitate has formed.

**Note.** It is important to mix thoroughly and gently after the addition of the Solution Y III to avoid localized precipitation of bacterial cell debris.

7. Centrifuge for 10 min at ~12,000 rpm to pellet cell debris and chromosomal DNA.
8. Carefully remove and load the supernatant from step 7 onto a spin column. Incubate at room temperature for 1~2 minutes. Centrifuge for 1 min at ~12,000 rpm. Discard the flow-through.
9. Wash the column once with 500 µl Wash Buffer DPB. Centrifuge at ~12,000 rpm for 1 min. Discard the flow-through.
10. Wash the column with 500 µl Wash Buffer DW, Centrifuge at ~12,000 rpm for 1 min. Discard the flow-through. Repeat Step 10 again.

**Note** Wash Buffer DW must previously diluted with 100% ethanol.

11. Centrifuge the column at ~12,000 rpm for 3 minute to remove any residual Wash Buffer DW. Discard the Wash Tube with the flow-through.
12. Discard the flow-through and centrifuge for an additional 1 min to remove residual Wash Solution. This step is essential to avoid residual ethanol in plasmid preps.
13. Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided), and pipet 30-100 µl Elution Buffer DTE (prewarm to 60°C) directly to the center of the column without touching the membrane. Incubate for 2 min at room temperature and centrifuge for 2 min at 14,000 × g (12,000rpm).

**Note.** An additional elution step (optional) with Elution Buffer or water will recover residual DNA from the membrane and increase the overall yield by 10-20%. For elution of plasmids or cosmids >20 kb, prewarm Elution Buffer to 60°C before applying to silica membrane.

14. Discard the column and store the purified plasmid DNA at -20°C.

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