TIANGEN®

Super Plant Genomic DNA Kit (Polysaccharides & Polyphenolics-rich)

For DNA purification from polysaccharides & polyphenolics-rich plants

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Super Plant Genomic DNA Kit (Polysaccharides & Polyphenolics-rich)

(Spin-column)

Cat. no. GDP360

Kit Contents

Contents	GDP360 (50 preps)
Buffer GPS	30 ml
Buffer GPA	10 ml
Buffer RD	12 ml
Buffer PW	15 ml
RNase A (10 mg/ml)	600 µl
RNase-Free Spin Columns CR2	50
Filtration Columns CS	50
Collection Tubes 2 ml	50
Buffer TB	15 ml
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Storage

This kit can be stored for 15 months in a dry environment under room temperature (15-30°C). If any precipitate forms in the buffers, it should be dissolved by warming the buffers at 37°C before use.

Introduction

This kit adopts specific DNA-binding spin-column and unique buffer system, which allows it to extract high-quality and pure genomic DNA from multiple plant tissues. Its unique precipitation solution can remove protein, polysaccharides and phenols from polysaccharide and polyphenol plants sample. The extracted genomic DNA fragment is highly pure and stable.

The gDNA purified by this kit can be applied to a variety of following operations, including enzyme digestion, PCR, library construction, Southern blotting, chip detection and high-throughput sequencing, etc.

Product Features

- **Easy and convenient:** Ultra-pure genomic DNA can be obtained within 1h.
- **Wide application:** It is applicable to multiple plant tissues, especially polysaccharide and polyphenol plants.
- **Safe and non-toxic:** No need for phenol/chloroform
- **Highly purified:** The obtained DNA has high purity and can be directly used in chip detection, high-throughput sequencing .

Important Notes <u>**Please read the notes before using this kit.**</u>

1. Avoid repeated freezing and thawing, otherwise the DNA fragments extracted will be smaller and the amount of extraction will be reduced.
2. If there's precipitation in Buffer GPS, please dissolve it at 37°C and shake it before use.

Reagents need to be prepared by Customer

96-100% ethanol

Protocol

Before use, please add ethanol (96%-100%) into Buffer RD and PW as indicated on the bottle.

1. Add liquid nitrogen in plant tissues and grind them thoroughly. Weigh about 100 mg of fresh plant tissues or 30 mg of dry mass tissues.
2. Immediately add 400 μl Buffer GPS and 10 μl RNase A (10 mg/ml) in the grinded powder. After vortex mixing, put the centrifuge tube in a 65°C water bath for 15 min. Turn the tubes several times to mix the sample in the water bath.

   Notes: If the solution turns thick after lysis, add more Buffer GPS , and increase the volume of Buffer GPA in step 3. The volume ratio of Buffer GPS and GPA shall be 4:1.
3. Add 100 μl Buffer GPA, votex for 1 min and centrifuge at 12,000 rpm (~13,400×g) for 5min. Transfer the supernatant solution to the Filtration Columns CS (**inside the Collection Tubes**), and centrifuge at 12,000 rpm (~13,400×g) for 1min before transferring the filtrate to a new microcentrifuge tube.

   Notes: If the solution turns thick after lysis, add Buffer GPA and vortex, then put the centrifuge tube on ice for 5min before performing centrifugation.
4. Add equal volume of ethanol (96%-100%) and mix thoroughly. Flocculent precipitation may appear.
5. Transfer all solutions and flocculent precipitations obtained last step to the RNase-Free Spin Columns CR2 (**inside the Collection Tube**). Centrifuge at 12,000 rpm (~13,400×g) for 1min and discard the flow-through. Then put the RNase-Free Spin Columns CR2 in the collection tube.
6. Add 550 μl Buffer RD (**check if the ethanol has been added**) to RNase-Free Spin Columns CR2. Centrifuge at 12,000 rpm (~13,400×g) for 1 min, discard the flow-through and then put the RNase-Free Spin Columns CR2 in the collection tube.
7. Add 700 μl Buffer PW (**check if the ethanol has been added**) to RNase-Free Spin Columns CR2 . Centrifuge at 12,000 rpm (~13,400×g) for 1 min, discard the flow-through and then put the RNase-Free Spin Columns CR2 in the collection tube.
8. Repeat Step 7.

9. Place the RNase-Free Spin Columns CR2 inside the collection tube, centrifuge at 12,000 rpm (~13,400×g) for 2min and transfer the RNase-Free Spin Columns CR2 to a new centrifuge tube. Dry it for 5-10 min at room temperature.

   Notes: Ethanol residue will inhibit the subsequent enzyme reaction, so make sure that the ethanol volatilizes completely when drying. But also don't dry for too long to avoid difficulty in eluting DNA.

10. Add 50-100 µl Buffer TB in the RNase-Free Spin Columns CR2 and incubate for 3-5 min at room temperature. Then centrifuge at 12,000 rpm (~13,400×g) for 2min and collect the eluent.

Measurement of DNA Concentration and Purity

The size of the genomic DNA fragment obtained is related to the sample preservation time, the shearing force during the operation and other factors. The concentration and purity of recovered DNA fragments can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer.

DNA should have a significant absorption peak at OD_{260}. If the OD_{260} value is 1, then it is equivalent to about 50µg/ml double-strand DNA and 40 µg/ml single-strand DNA.

The OD_{260}/OD_{280} ratio should be 1.7-1.9. If it is not the elution buffer but deionized water is used, the ratio will be low, because the pH value and the presence of ions will affect the light absorption value, but it does not mean the purity is low.