EasyPrep Polyphenol Plant DNA Extraction Kit

For purification of DNA from high-polyphenol plants

Contents

Introduction

EasyPrep Polyphenol Plant DNA Extraction Kit 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 purify genomic DNA from a wide variety of plant species and tissues with polyphenol contents, and the whole process is completed in <1 h. Purified DNA is suitable for PCR, restriction endonuclease digestion, and Southern hybridization.

Yield of Genomic DNA with EasyPrep Plant DNA Kit

Sample	Wet weight	DNA Yeild
Plant tissues	100 mg	3–30 μg

Note: DNA yields vary due to genome size, ploidy, age of sample, etc. Material collected as young leaves or needles give the best results.

Important Note

1. Repeated freezing and thawing should be avoided as this reduces the DNA size and quantity.
2. If a precipitate has formed in Buffer GBP1 or Buffer GBP2, heat the buffer to 56 °C until the precipitate has fully dissolved.
3. All steps should be carried out at room temperature (15–25 °C).

Kit Contents

Contents	DPT-BC05 50 preps	DPT-BC05-2 200 preps
Buffer GBP1	40 mL	160 mL
Buffer GBP2	40 mL	160 mL
Buffer GBD	13 mL	52 mL
Buffer PBW	15 mL	50 mL
Buffer TBE	15 mL	60 mL
Spin Columns CB3	50	200
Collection Tubes（2 ml）	50	200

Storage

EasyPrep Polyphenol Plant DNA Extraction Kit should be stored dry at room temperature (15–25 °C) up to 12 months.

Protocol

Ensure that ethanol (96%−100%) has been added into Buffer GBD and Buffer PBW before use

1. Place 100 mg wet weight of plant tissue or 30 mg of lyophilized plant tissue, and grind the samples thoroughly in liquid nitrogen.
2. Add 700 µL of 65 °C preheated GBP1 (β-mercaptoethanol (β-ME) must be added to Buffer GBP1 before use. The final concentration of β-ME is 0.1%.) to the powdered plant tissue, and vortex for 10−20 sec to mix, making sure to disperse all clumps. Next, incubate for 20 min at 65 °C, and mix by inverting the tube several times.
3. Add 700 µL of chloroform, mix by inverting the tube for several times, and centrifuge for 5 min at 12,000 rpm (~13,400×g).
4. Pipette the supernatant to a new tube, add 700 µL of Buffer GBP2, and mix by inverting the tube several times.
5. Pipette all of the mixture from Step 4, including any precipitate that may have formed, into Spin Column CB3 (place Spin Column CB3 in the collection tube). Close the CB3 lid, and centrifuge for 30 sec at 12,000 rpm (~13,400×g). Discard the filtrate, and set the spin column CB3 into the collection tube.

Note: Because the capacity of CB3 is 700 µL, the loading-centrifugation step should be repeated several times for processing all the mixture from Step 4.

6. Carefully open the column, and add 500 µL of Buffer GBD (Ensure that ethanol (96%−100%) is added to Buffer GBD before use). Close the lid, and centrifuge at 12,000 rpm (~13,400×g) for 30 sec. Discard the filtrate, and place the spin column into the collection tube.
7. Add 700 µL of Buffer PBW (Ensure that ethanol is added to Buffer PBW before use) to Spin Column CB3 to wash the membrane, and centrifuge for 30 sec at 12,000 rpm (~13,400×g). Discard the flow-through, and replace Spin Column CB3 in the collection tube.
8. Add 500 µL of PBW (Ensure that ethanol (96%−100%) is added to Buffer PBW before use) to the Spin Column CB3 to wash the membrane, and centrifuge for 30 sec at 12,000 rpm (~13,400×g). Discard the flow-through, and replace Spin Column CB3 in the collection tube.
9. Replace Spin Column CB3 in the collection tube, and centrifuge for 2 min at 12,000 rpm (~13,400×g) to remove residual Buffer PBW. Discard the collection tube, and transfer Spin Column CB3 to a clean 1.5-mL or 2-mL microcentrifuge tube. Open the lid of CB3, and incubate the assembly at room temperature (15–25 °C) or 50 °C for several minutes to dry membrane completely.

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

10. Pipette 50–200 μL of Buffer TBE directly onto the CB3 membrane, incubate for 2−5 min at room temperature (15–25 °C), and then centrifuge for 2 min at 12,000 rpm (~13,400×g) to elute.

Note: Ensure that the Elution Buffer TBE is equilibrated to room temperature (15–25 °C). Elution with small volumes (<50 μL) will reduce the elution efficiency and the DNA yield. The pH value of the eluted buffer will have some influence on elution; Buffer TBE or distilled water (pH 7.0–8.5) is recommended for DNA elution. For long-term storage of DNA, eluting in Buffer TBE and storing at –20 °C is recommended because DNA stored in water is subject to acid hydrolysis.