EasyPrep Plant DNA Extraction Kit

For isolation of gDNA from plants

Contents

Introduction

EasyPrep Plant DNA Extraction Kit provides a fast, simple, and cost-effective gDNA miniprep method for routine molecular biology laboratory applications. The kit uses silica membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. DNA binds to the silica membrane while contaminants such as proteins and polysaccharides are efficiently removed by two wash steps. EasyPrep Plant DNA Purification Kit is ready to use and can be applied to purify gDNA from various plant species and tissues, and the whole process is <1 h. Furthermore, phenol extraction and ethanol precipitation are not required. Purified DNA is suitable for PCR, restriction digestion, gDNA library construction, and Southern hybridization.

Important Notes

1. Do not freeze-thaw the samples repeatedly. Otherwise, DNA size will decrease and a lower DNA yield will be produced.
2. Buffer LBP1 may become slightly yellow upon storage. This does not affect the result.
3. If precipitates form in Buffer LBP1 or Buffer LBP2, dissolve them by incubating at 56 °C.
4. All centrifuge steps should be performed at room temperature (15–25 °C).

Kit Contents

Contents	DPT-BC20 50 preps	DPT-BC20 200 preps
RNase A (10 mg/ml)	300 µL	1.25 mL
LBP1 Buffer	25 mL	100 mL
LBP2 Buffer	10 mL	40 mL
LBP3 Buffer	21 mL	84 mL
PBW Buffer	15 mL	50 mL
TBE Buffer	15 mL	60 mL
RNase-free Spin Columns CB3	50	200
RNase-free Collection Tubes（2 ml）	50	200

Storage

EasyPrep Plant DNA Extraction Kit should be stored dry at room temperature (15–25 °C) and is stable for 12 months.

Protocol

Ensure that ethanol (96%–100%) has been added to Buffer LBP3 and Buffer PBW as indicated on the tag of the bottle before use.

1. Take 100 mg (wet weight) of plant tissue or 20 mg of lyophilized plant tissue and grind it thoroughly in liquid nitrogen. Add 400 µL of Buffer LBP1 and 6 µL of RNase A (10 mg/mL) to the powder plant tissue. Mix it on a vortex mixer for 1 min. Disperse all clumps and then incubate for 10 min at room temperature (15–25 °C).
2. Add 130 µL of Buffer LBP2 to the lysate and mix thoroughly on a vortex mixer for 1 min.
3. Centrifuge for 5 min at 12,000 rpm (~13,400 ×g). Pipet the supernatant to a clean 1.5-mL microcentrifuge tube.
4. Add 1.5 times volume of Buffer LBP3; for example, for 500 µL flow-through, add 750 µL Buffer LBP3 (ensure that ethanol has been added to Buffer LBP3 before use). Mix immediately on a vortex mixer for 15 sec. Precipitates may form after the addition of Buffer LBP3.
5. Pipet the mixture from Step 4, including any precipitates that may have formed, into Spin Column CB3 (place Spin Column CB3 in the collection tube). Centrifuge for 30 sec at 12,000 rpm (~13,400 ×g), and discard the flow-through. Place Spin Column CB3 back into the collection tube.
6. Add 700 µL of Buffer PBW to Spin Column CB3 to wash the membrane (ensure that ethanol has been added to Buffer PBW before use), centrifuge for 30 sec at 12,000 rpm (~13,400 ×g), and discard

the flow-through. Place Spin Column CB3 back into the collection tube.

Note: If the membrane remains substantially colored (dark green or yellow), add 500 µL of ethanol into Spin Column CB3. Centrifuge for 30 sec at 12,000 rpm (~13,400 ×g) and discard the flow-through. Place Spin Column CB3 back into the collection tube.

7. Add 500 µL of Buffer PBW to Spin Column CB3 to wash the membrane, and centrifuge for 30 sec at 12,000 rpm (~13,400 × g). Discard the flow-through.
8. Place Spin Column CB3 back into the collection tube, and centrifuge for 2 min at 12,000 rpm (~13,400 ×g) to remove residual Buffer PBW. Open the lid of Spin Column CB3 and incubate the assembly at room temperature (15–25 °C) for several minutes to dry the membrane completely.

Note: Residual ethanol from Buffer PBW inhibits subsequent enzymatic reactions (e.g., enzyme cleavage and PCR).

9. Discard the collection tube and transfer Spin Column CB3 to a clean 1.5-mL microcentrifuge tube. Pipet 50–200 µL Buffer TBE directly onto Spin Column 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: If the volume of the eluted buffer is <50 µL, the recovery efficiency may decrease. The pH of the elution buffer influences eluting; we suggest using Buffer TBE or distilled water (pH 7.0–8.5) to elute gDNA. 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.