

EasyPure® Plant Genomic DNA Kit

Cat. No. EE111

Storage: RNase A at -20°C for two years; others at room temperature (15-25°C) for one year

Description

EasyPure® Plant Genomic DNA Kit provides a simple and convenient way to isolate high quality plant genomic DNA from plant tissues (up to 100 mg). Plant tissue is disrupted by grinding in liquid nitrogen, and DNA is released with detergent. Proteins, polysaccharides, and cell debris are eliminated by precipitation. DNA is bound to silica-based column. The isolated genomic DNA is suitable for PCR, restriction enzyme digestion and southern blotting.

- DNA yield up to 15 µg.
- Complete removal of pigment, polysaccharides and other impurity.
- DNA range of 20-50 kb.
- Column based purification, no organic extraction or ethanol precipitation.

Kit Contents

Components	EE111-01/11 (50 rxns)	EE111-02/12 (200 rxns)
Resuspension Buffer1 (RB1)	15 ml	60 ml
Precipitation Buffer1 (PB1)	6 ml	25 ml
Binding Buffer1 (BB1)	8 ml	32 ml
Clean Buffer 1 (CB1)	30 ml	110 ml
Wash Buffer 1 (WB1)	12 ml	2×22 ml
Elution Buffer (EB)	25 ml	80 ml
RNase A (10 mg/ml)	800 µl/-	4×800 µl/-
Genomic Spin Columns with Collection Tubes	50 each	200 each

Procedures

Before starting, adding appropriate volume of 96-100% ethanol to BB1 and WB1.

Components	EE111-01/11 (50 rxns)	EE111-02/12 (200 rxns)
Binding Buffer 1 (BB1)	12 ml	48 ml
Wash Buffer 1 (WB1)	48 ml	2×88 ml

All centrifugation steps are carried out at room temperature.

1. Grind 100 mg fresh plant tissues or 20 mg hard plant tissues in liquid nitrogen to a powder.
2. Add 250 µl RB1 and 15 µl RNase A to the powder. Mix thoroughly by vortexing to completely suspend the sample.
3. Incubate at 55°C in water bath for 15 minutes.
4. Centrifuge at 12,000 rpm for 5 minutes, gently transfer the supernatant to a sterile microcentrifuge tube.
5. Add 100 µl PB1, mix thoroughly by vortexing, incubate on ice for 5 minutes, centrifuge at 12,000 rpm for 5 minutes.
6. Gently transfer the supernatant to a sterile microcentrifuge tube, add 375 µl BB1 (check to ensure you have added ethanol before use). Mix thoroughly.
7. Apply all the lysate to a spin column, centrifuge at 12,000 rpm for 30 seconds, discard the flow-through.

8. Add 500 μ l CB1, centrifuge at 12,000 rpm for 30 seconds, and discard the flow-through.
9. Add 500 μ l WB1 (check to ensure you have added ethanol before use), centrifuge at 12,000 rpm for 30 seconds, discard the flow-through.
10. Repeat step 9 once.
11. Centrifuge at 12,000 rpm for 2 minutes to remove residual WB1.
12. Place the spin column in a sterile 1.5 ml microcentrifuge tube. Add 50-200 μ l of Elution Buffer (preheated to 60°C, able to increase DNA yield) or distilled water (pH >7.0) to the center of column. Incubate at room temperature for 1 minute. Centrifuge at 12,000 rpm for 1 minute to elute the genomic DNA (to recover more DNA, add EB or distilled water again to perform a second elution). For long-term storage, store the purified DNA at -20°C

Notes

- Do not use too many starting materials in case it affect the extraction performance.
- It is important not to overload the column, as this can lead to significantly lower yields than expected.
- Use sterile tubes and pipette tips to avoid the contamination from DNase.
- You may perform the second elution step using the same microcentrifuge tube or different tubes.

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