

Instructions for Use

Revision date: 17-Sep-20

Plant Genomic DNA Kit (No RNase A)

Catalog No.: abx098863

Size: 50 rxns / 200 rxns

Storage: Store the kit components at room temperature (15-25 °C) for up to one year.

Introduction

Abbexa's 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 and DNA is isolated using a silica-based column. The isolated genomic DNA is suitable for PCR, restriction enzyme digestion and southern blotting.

Kit Components

Reagent	50 rxns	200 rxns
Resuspension Buffer	15 ml	60 ml
Precipitation Buffer	6 ml	25 ml
Binding Buffer	8 ml	32 ml
Clean Buffer	30 ml	110 ml
Wash Buffer	12 ml	2 x 22 ml
Elution Buffer	25 ml	80 ml
Genomic Spin Columns with Collection Tubes	50	200

Material Required But Not Provided

RNase A (10 mg/ml, stored at -20 °C)

Protocol

Reagent Preparation

- **Working Binding Buffer solution:** Dilute the Binding Buffer with 96-100% ethanol to a ratio of 2:1 (i.e. add 8 ml of Binding Buffer into 4 ml of 96-100% ethanol to form 12 ml of Working Binding Buffer solution).
- **Working Wash Buffer solution:** Dilute the Wash Buffer with 96-100% ethanol to a ratio of 1:3 (i.e. add 12 ml of Wash Buffer into 36 ml of 96-100% ethanol to form 48 ml of Working Wash Buffer solution).

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Assay Procedure

1. Grind up to 100 mg of fresh plant tissues or 20 mg of hard plant tissues in liquid nitrogen to a powder.
2. Add 250 μ l of Resuspension Buffer and 15 μ l of RNase A (10 mg/ml, not provided with the kit) to the powder. Mix thoroughly by vortexing to completely suspend the sample.
3. Incubate at 55 °C in a water bath for 15 minutes.
4. Centrifuge at 12,000 RPM for 5 minutes, then carefully transfer the supernatant to a sterile microcentrifuge tube.
5. Add 100 μ l of Precipitation Buffer to the supernatant and mix thoroughly by vortexing. Allow to stand on ice for 5 minutes, then centrifuge at 12,000 RPM for 5 minutes. Carefully transfer the supernatant to a sterile microcentrifuge tube.
6. Add 375 μ l of Working Binding Buffer solution (with ethanol) to the supernatant and mix thoroughly.
7. Transfer the lysate to a spin column and centrifuge at 12,000 RPM for 30 seconds. Discard the flow-through.
8. Add 500 μ l of Clean Buffer and centrifuge at 12,000 RPM for 30 seconds. Discard the flow-through.
9. Add 500 μ l of Working Wash Buffer solution (with ethanol) and centrifuge at 12,000 RPM for 30 seconds. Discard the flow-through.
10. Repeat Step 9 above once more.
11. Centrifuge at 12,000 RPM for 2 minutes to remove residual Working Wash Buffer solution.
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 to increase DNA yield) or distilled water (pH > 7.0) to the center of the column. Allow to stand at room temperature for 1 minute.
13. Centrifuge at 12,000 RPM for 1 minute to elute the genomic DNA. To recover more DNA, add Elution Buffer or distilled water again to perform a second elution. The second elution can be performed using the same microcentrifuge tubes or new sterile tubes. The purified DNA can be stored at -20 °C for long-term storage.

Notes

- Exceeding the sample weight in Step 1 may affect extraction performance.
- Do not overload the column, as this may lead to a significantly lower yield than expected.
- Use sterile tubes and pipette tips to avoid DNase contamination.