Ver: 1912

Plant gDNA Isolation Kit

（BW-GD2611）

Contents

Kit Contents

Catalog#	BW-GD2611-00	BW-GD2611-01	BW-GD2611-02
Preps	4	50	250
DNA Mini Columns	4	50	250
2 mL Collection Tubes	4	50	250
Buffer P1	4 mL	50 mL	250 mL
Buffer P2	1 mL	8 mL	40 mL
Buffer P3	2 mL	20 mL	100 mL
DNA Wash Buffer*	2 mL	15 mL	3 x 24 mL
Elution Buffer	1 mL	8 mL	40 mL
RNase A（20 mg/mL）	25 µL	270 µL	1.4 mL
User Manual	1	1	1

*Add 8 mL (BW-GD2611-00) or 60 mL (BW-GD2611-01) or 96 mL (BW-GD2611-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

Introduction

The Plant gDNA Isolation Kit provides a fast and easy method for isolating high-quality gDNA from various plant tissues. The kit uses a unique buffer system and binding matrix, which allow efficient and specific binding of nucleic acid to silica membranes. Plant tissue of 100 mg fresh (or 50 mg dry sample) can be processed within 1 hour.

The purified DNA can be directly used in downstream molecular experiments such as PCR, Southern hybridization, and restriction enzyme digestion. The entire extraction process does not require phenol-chloroform extraction and multiple samples can be processed simultaneously.

Storage and Stability

All Plant gDNA Isolation Kit components are guaranteed for at least 12 months from the date of production when stored as follows:

- RNase A is stable at room temperature for 12 months. For long-term, stored RNase A at 4°C.
- Store all other materials at room temperature (15-25°C) .

- If precipitates form in Buffer P1 and Buffer P2, dissolve the precipitates at 65℃ before use.

Before Starting

The isolation protocol is simple, fast, and reliable provided that all steps are followed diligently. Please read the entire booklet and get all necessary supplies and equipments.

Important Notes

Dilute DNA Wash Buffer with absolute ethanol as follows: Add 8 mL (BW-GD2611-00) or 60 mL (BW-GD2611-01) or 96 mL (BW-GD2611-02) of absolute ethanol to each bottle. The final concentration is 80%.

Materials not Supplied

- Tabletop centrifuge
- Sterile 1.5 mL and 2.0 mL centrifuge tubes
- Water bath
- Absolute ethanol

Safety Information

Buffer P3 contains chaotropic salts, which may form reactive compounds when combines with bleach, do not add bleach or acidic solutions directly to the preparation waste, wear gloves and protective eyewear when handling.

Plant DNA Isolation Protocols

This procedure applies to <100 mg fresh plant tissue, or < 50 mg dry plant tissue. Before the experiment, please adjust the water bath to 65℃ and preheat the Elution Buffer.

1. A: Fresh plant tissue:

   Take fresh plant tissue (not more than 100 mg), place it in a mortar, add **900 µL** of **Buffer P1**, grind at room temperature, and grind until there is no visible particulate suspension visible to the naked eye.

   B: Dry plant tissue:

   Take dry tissue (not more than 50 mg), place in a mortar, add **950 µL** **Buffer P1**, grind it at room temperature, and grind it until there is no obvious particle suspension (If the dry tissue is difficult to grind, let it soak in the buffer for 10-20 minutes before processing).

2. After grinding, transfer the mixture to a 2 mL centrifuge tube, vortex immediately and place it in a 65℃ water bath for 10 minutes (dry tissue may be extended as appropriate).

   Optional: If RNA removal is required, add **5 µL** of **RNase A** before warming.

3. Add **140 µL** **Buffer P2** and mix by vortexing for 10 seconds. Centrifuge at 12,000 × g for 10 minutes.

4. Carefully pipette the supernatant into a new 2 mL centrifuge tube, avoid disturbing the pellet. Add **0.5 volume** of **Buffer P3** and 0.5 volume of absolute ethanol, mix well by vortexing for 10 seconds.

5. Insert a **DNA Mini Column** into a **2 mL Collection Tube**, and add the sample to the column. Centrifuge at 12,000 ×g for 30 seconds. Discard the flow-through liquid and insert the column back into the collection tube.

6. Add **600 µL** of **DNA Wash Buffer**, centrifuge at 12,000 ×g for 30 seconds, discard the flow-through liquid and re-insert the column into the collection tube.

7. Add another **600 µL** of **DNA Wash Buffer**, centrifuge at 12,000 ×g for 30 seconds. Discard flow-through liquid.

8. Put the empty column, **with the lid open**, into the same collection tube and centrifuge at 12,000 ×g for 2 minutes.

   Note： Residual ethanol will be removed more efficiently with the lid of the column open. It is critical to remove the ethanol before elution.

9. Insert the **DNA Mini Column** into a 1.5 mL centrifuge tube and add **100-150 µL** of pre-heated (65℃) **Elution Buffer** to the column. Let stand for 2 minutes at room temperature. Centrifuge at 12,000 ×g for 1 minute to elute DNA.

   Optional: Add the eluted DNA back to the column for a second elution normally yields another 20-30% of the DNA while the first elution typically yields 60-70% of the DNA.

Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl Buffer, or Elution Buffer as a blank. Dilute the DNA in TE buffer and calculate the concentration using the following equation:

[DNA] = (Absorbance 260) × (0.05 µg/µL) × (Dilution Factor)

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A $_{260/280}$ ratio of 1.7-1.9 corresponds to 85%-95% purity.

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Clogged column	Carry-over of debris.	Make sure no particulate material is transferred.
	Sample to viscous.	Do not exceed suggested amount of starting material. Alternatively, increase amounts of Buffer P1 and P2 and use two or more columns per sample.
Low DNA yield	Incomplete disruption of starting material.	For both dry and fresh samples, obtain a fine homogeneous powder before adding Buffer P1.
	Poor lysis of sample.	Decrease amount of starting material or increase amount of Buffers P1, P2 and P3.
	DNA remains bound to column.	Increase elution volume to 200 µL and incubate on column at 65°C for 5 minutes before centrifugation.
	DNA washed off.	Dilute DNA Wash Buffer Concentrate by adding appropriate volume of absolute ethanol prior to use (page 3).
Problems in downsteam applications	Salt carry-over.	DNA Wash Buffer must be at room temperature.
	Ethanol carry-over	Following the second wash spin, ensure that the column is dried by centrifuging 2 minutes at maximum speed.

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us or visit our website.

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