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# CP Plant gDNA Isolation Kit

# （BW-GD2621）

## Contents

## Kit Contents

| Catalog# | BW-GD2621-00 | BW- GD2621-01 | BW- GD2621-02 |
|---|---|---|---|
| Preps | 4 | 50 | 250 |
| DNA Mini Columns | 4 | 50 | 250 |
| 2 mL Collection Tubes | 4 | 50 | 250 |
| Buffer CP1 | 2.1 mL | 26 mL | 130 mL |
| Buffer CP2 | 1 mL | 8 mL | 40 mL |
| DNA Wash Buffer* | 2 mL | 15 mL | 3 x 24 mL |
| RNase A (20 mg/mL) | 25 µL | 270 µL | 1.4 mL |
| Elution Buffer | 1 mL | 8 mL | 40 mL |
| User Manual | 1 | 1 | 1 |

*Add 8 mL (BW-GD2621-00) or 60 mL (BW-GD2621-01) or 96 mL (BW-GD2621-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

## Introduction

The CP Plant gDNA Isolation Kit is designed for efficient recovery of genomic DNA from fresh and dried plant tissue samples rich in polysaccharides and other secondary metabolites. Up to 100 mg of wet tissue (or 50 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of the matrix with the speed and versatility of spin column technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant tissue lysates. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques.

If using the CP Plant gDNA Isolation Kit for the first time, please read this booklet to become familiar with the procedures. This procedure relies on the well established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the selective DNA binding of Biomiga's ezBind matrix. Samples are homogenized and lysed in a high salt buffer containing CTAB and extracted with chloroform to remove polysaccharides and other components that interfere with many DNA isolations and downstream applications. Binding

conditions are then adjusted and DNA is further purified using DNA Mini Columns. In this way salts, proteins and other contaminants are removed to yield high quality genomic DNA suitable for downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization.

## Storage and Stability

All CP Plant gDNA Isolation Kit contents 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℃.

✪ Store all other materials at room temperature (15-25℃）.

## Before Starting

Prepare all components and get all necessary materials ready by examining this user manual and become familiar with each step.

## Important Notes

✪ Add 8 mL (BW-GD2621-00) or 60 mL (BW-GD2621-01) or 96 mL (BW-GD2621-02) 96-100% ethanol to each DNA Wash Buffer bottle before use.

✪ Choose the most appropriate protocol to follow. Procedures are described for each of dried and fresh (or frozen) specimens. In addition, a short protocol is given for isolation of DNA for PCR reactions.

| | |
|---|---|
| **A. Dry Specimens** | For processing ~50 mg powdered tissue. |
| **B.Fresh/Frozen Specimens** | For processing ≤100 mg fresh (or frozen) tissue. Yield is similar to A. |

✪ If precipitates form in Buffer CP1 and Buffer CP2, dissolve the precipitates at 65℃ before use.

✪*Carry out all centrifugations at room temperature.*

## Materials not Supplied

✪ Microcentrifuge capable of at least 14,000 ×g.

✪ Nuclease-free 1.5 mL and 2.0 mL centrifuge tubes.

✪ chloroform:isoamyl alcohol (24:1).

✪ Waterbath equilibrated to 65℃.

✪ Equilibrate sterile dd$H_2O$ or Elution Buffer at 65℃.

✪ Absolute (96%-100%) ethanol.

✪ Optional: β-mercaptoethanol.

✪ Liquid nitrogen for freezing/disrupting samples.

**Note:** Use extreme caution when handling liquid nitrogen.

## Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets.

# CP Plant gDNA Protocol (For spin)

## A. Dry Specimens

This is the most robust method for isolation of total cellular (mitochondrial, chloroplast, and genomic) DNA. Yields are usually sufficient for several tracks on a Southern blot for RFLP mapping.

Drying allows storage of field specimens for prolonged periods of time prior to processing. Samples can be dried overnight in a 45℃ oven, powdered, and stored dry at room temperature. To prepare dried samples, place ~50 mg of dried tissue into a 2.0 mL microfuge tube and grind using a pellet pestle. For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until clean. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and wiping the surface clean between samples. A fine powder will ensure optimal DNA extraction and yield. Process in sets of four to six tubes until step **2** before starting another set.

1. For 10-50 mg powdered dry tissue, add **500 µL Buffer CP1** in a 2.0 mL microfuge tube and vortex vigorously to mix. Make sure to disperse all clumps.
2. Incubate at 65℃ for 15 minutes. Mix sample twice during incubation by inverting tube. **Optional:** If necessary, add **5 µL** of **RNase A** into the lysate before incubation to remove the RNA.
3. Add 600 µL chloroform /Isoamyl alcohol (24:1) and vortex to mix. Centrifuge at ≥10,000 ×g for 10 minutes.
4. Carefully aspirate **300 µL** supernatant to a new 1.5 mL microfuge tube, make sure not to disturb the pellet or transfer any debris.
5. Adjust binding conditions of the sample by adding **150 µL Buffer CP2** followed by 300 µL absolute ethanol and vortex to obtain a homogeneous mixture. A precipitate may form upon addition of ethanol, it will not interfere with DNA isolation.
   **Note:** This point to start the optional vacuum/spin protocol. (See page 7 for details.)
6. Insert a **DNA Mini Column** into a **2 mL Collection Tube**. Apply the entire sample

(including any precipitate that may have formed) to the Column. Centrifuge the column at 12,000 ×g for 1 minute to bind DNA. Discard the flow- through liquid. Reuse the collection tube in next step below.

7. Add **600 µL DNA Wash Buffer** diluted with absolute (96%-100%) ethanol. Centrifuge at 12,000 ×g for 1 minute and discard the flow-through liquid. Reuse the collection tube in next step below.

   **Note:** DNA Wash Buffer must be diluted with absolute (96%-100%) ethanol prior to use. Follow directions on label.

8. Repeat wash step with an additional **600 µL DNA Wash Buffer**. Centrifuge at 12,000 ×g for 1 minute. Discard flow-through.
9. Replace the column into the collection tube and centrifuge empty column, with the lid open, for 2 minutes at maximum speed to dry. This step is critical for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.
10. Transfer column to a clean 1.5 mL tube. Apply **100 µL Elution Buffer** pre-heated to 65℃ and immediately centrifuge at maximum speed for 1 minute.

## B. Fresh/Frozen Specimens

This protocol is suitable for most fresh or frozen tissue samples allowing more efficient recovery of DNA. However, due to the tremendous variation in water and polysaccharide content of plants, sample size should be limited to ≦ 200 mg. Best results are obtained with young leaves or needles. The method isolates sufficient DNA for several tracks on a standard Southern assay.

To prepare samples collect tissue in a 1.5 mL or 2 mL microfuge tube and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Alternatively, one can allow liquid nitrogen to evaporate and then store samples at -70℃ for later use. For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until cleaning. Disposable pestles may be autoclaved several times. For standard southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and carefully wiping the surfaces clean between samples.

1. Collect ground plant tissue (start with 100 mg) in a 2.0 mL microfuge tube and immediately

add **500 µL Buffer CP1** and vortex vigorously. Make sure to disperse all clumps. DNA cannot be effectively extracted from clumped tissue.

2. Incubate at 65℃ for 15 minutes. Mix sample twice during incubation by inverting the tube.

   **Optional:** If necessary, add **5 µL** of **RNase A** into the lysate before incubation to remove the RNA.

3. Add 800 µL chloroform /Isoamyl alcohol (24:1) and vortex to mix. Centrifuge at ≥10,000 ×g for 5 minutes.

4. Carefully aspirate **300 µL** supernatant to a new 1.5 mL microfuge tube making sure not to disturb the pellet or transfer any debris.

5. Adjust binding conditions of the sample by adding **150 µL Buffer CP2** followed by 300 µL absolute ethanol and vortex to obtain a homogeneous mixture. A precipitate may form upon addition of ethanol, it will not interfere with DNA isolation.

   **Note:** This point to start the optional vacuum/spin protocol. (See page 7 for details.)

6. Apply the entire sample (including any precipitate that may have formed) to a **Mini Column** placed in a **2 mL Collection Tube** (supplied). Centrifuge the column at 12,000 ×g for 1 minute to bind DNA. Discard the flow-through liquid.

7. Add **600 µL DNA Wash Buffer** to the column. Centrifuge at 12,000 ×g for 1 minute and discard the flow-through liquid. Reuse the collection tube in next step below.

   **Note:** DNA Wash Buffer must be diluted with absolute (96%-100%) ethanol prior to use. Follow directions on label.

8. Repeat wash step with **600 µL DNA Wash Buffer**. Centrifuge at 12,000 ×g for 1 minute. Discard flow-through.

9. Put the column back to collection tube and centrifuge the empty column for 2 minutes at maximum speed. This step is critical for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.

10. Transfer the column to a clean 1.5 mL tube. Apply **100 µL Elution Buffer**, pre-warmed to 65℃ and incubate at room temperature for 1 minute. 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.

# CP Plant gDNA Protocol (For spin/vacuum)

**Note:** Please read through previous section of this manual before using this protocol.

1. Prepare wet or dry samples by following the standard protocol in previous sections until loading DNA/CP2/Ethanol mixture to **DNA Mini Column.**
2. Prepare the vacuum manifold according to manufacturer's instruction and connect the Mini Column to the manifold.
3. Load the DNA/CP2/Ethanol solution to the column.
4. Switch on vacuum source to draw the sample through the column and turn off the vacuum.
5. Wash the column by adding **600 µL DNA Wash Buffer**, draw the wash buffer through the column by turn on the vacuum source. Repeat this step with another **600 µL DNA Wash Buffer**.
6. Assemble the column into a **2 mL Collection Tube** and transfer the column to a micro centrifuge. Spin 1 minute to dry the column.
7. Place the column in a clean 1.5 mL microcentrifuge tube and add **100 µL Elution Buffer**. Centrifuge at maximum speed for 1 minute to elute DNA.

## Trouble Shooting Guide

| Problems | Possible Reasons | Suggested Improvements |
|---|---|---|
| Clogged column | Carry-over of debris. | Following extraction with chloroform: isoamyl alcohol, make sure no particulate material is transferred. |
| | DNA pellet not completely dissolved before applying sample to column. | In Protocols A and B, ensure that DNA is dissolved in water before adding Buffer CP2 and ethanol. This may need repeated incubation at 65℃ and vortexing. |
| | Sample too viscous. | Do not exceed suggested amount of starting material. Alternatively, increase amounts of Buffers CP1 and CP2 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 CP1. |
| | Poor lysis of sample. | Decrease amount of starting material or increase amount of Buffer CP1, chlorosoamyl alcohol, and Buffer CP2. |
| | DNA remains bound to column. | Increase elution volume to 200 µL and incubate on column at 65℃ for 5 minutes before centrifugation. |
| | DNA washed off. | Dilute DNA Wash Buffer by adding appropriate volume of absolute ethanol prior to use (page 3). |
| Problems in downstream 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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