Ver: 1912

Fungal gDNA Isolation Kit

（BW-GD2416）

Contents

Kit Contents

Catalog#	BW-GD2416 -00	BW-GD2416 -01	BW-GD2416 -02
Preps	4	50	250
DNA Mini Columns	4	50	250
2 mL Collection Tubes	4	50	250
Buffer FG1	3 mL	35 mL	160 mL
Buffer FG2	1 mL	8 mL	40 mL
Buffer FG3	2 mL	20 mL	100 mL
RNase A (20 mg/mL)	25 µL	270 µL	1.4 mL
DNA Wash Buffer*	2 mL	15 mL	3 x 24 mL
Elution Buffer	1.5 mL	15 mL	50 mL
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*Add 8 mL (BW-GD2416-00) or 60 mL (BW-GD2416-01) or 96 mL (BW-GD2416-02) absolute (96%-100%) ethanol to each bottle.

Introduction

Fungal gDNA Isolation Kits allow rapid and reliable isolation of high-quality total cellular DNA from a wide variety of fungal species and tissues. Up to 100 mg of wet tissue (or up to 50 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of Biomiga's matrix with the speed and versatility of spin column technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from fungal tissue lysates. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel.

If using the Fungal gDNA Isolation Kit for the first time, please read this manual to become familiar with the procedures before beginning. Dry or fresh fungal tissue is disrupted and then lysed in a specially formulated buffer containing detergent. Proteins, polysaccharides, and cellular

debris are subsequently precipitated. Binding conditions are then adjusted and the sample is applied to a Mini Column. Two rapid wash steps remove trace contaminants such as residual polysaccharides, and pure DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

Storage and Stability

All components of the Fungal gDNA Isolation Kit are stable for at least 12 months from date of production when stored at room temperature (15-25℃). RNase A is stable at room temperature, for long-term, stored RNase A at 4℃. During shipment or storage in cool ambient conditions, precipitates may form in Buffer FG1 and FG3, dissolve the precipitates at 50℃ before use.

Before Starting

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

Important Notes

☑ Preheat Elution Buffer to 65℃.

☑ Dilute DNA Wash Buffer concentrate with ethanol as follows and store at room temperature. Add 8 mL (BW-GD2416-00) or 60 mL (BW-GD2416-01) or 96 mL (BW-GD2416-02) absolute (96%-100%) ethanol to each bottle.

☑ Choose the most appropriate protocol to follow. Procedures are described for each of dried and fresh (or frozen) specimens.

A. Dry Specimens For processing ≤ 50 mg powdered tissue. Yield is sufficient for several tracks on southern assay.

B. Fresh or Frozen For processing ≤ 100 mg fresh (or frozen) powdered tissue.

Fungal gDNA Isolation Protocol

A. Dry Specimens

Materials supplied by users:

- Centrifuge capable of at least 10,000 xg.
- Nuclease-free 1.5 mL or 2.0 mL microfuge tubes.
- Waterbath equilibrated to 65 ℃.
- Equilibrate sterile ddH_2O water at 65 ℃.
- Absolute (96%-100%) ethanol.
- Paper towels.

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 or 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 microfuge (2 mL tubes are recommended for processing of >50 mg tissue) tube and grind using a pellet pestle. Disposable Kontes pestles work well. 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.

1. To **10-50 mg powdered dry tissue,** add **600 µL Buffer FG1.** Vortex vigorously to mix. Make sure to disperse all clumps.

   Tip: Process in sets of four to six tubes: grind, add Buffer FG1, then proceed to step **2** before starting another set. Do not exceed 50 mg dried tissue.

2. Incubate at 65℃ for 10 minutes. Mix sample twice during incubation by inverting tube.

   Optional: If necessary, add **5 µL** of **RNase A(20 mg/mL)** into the lysate before incubation to remove the RNA.

3. Add **140 µL Buffer FG2** and vortex to mix. Incubate on ice for 5 minutes. Centrifuge at ≥10,000 x g for 10 minutes.

4. Carefully aspirate supernatant to a new microfuge tube, making sure not to disturb the pellet or transfer any debris. Add **0.5 volume Buffer FG3** followed by 1 volume absolute ethanol and vortex to obtain a homogeneous mixture. A precipitate may form upon addition of ethanol(It doesn't interfere with DNA isolation).
5. Insert a **DNA Mini Column** into a **2 mL Collection Tube**. Apply the entire sample (including any precipitate that may have formed) to the **DNA Mini Column**. Centrifuge the column at 10,000 x g for 1 minute to bind DNA. Discard the flow-through liquid. Reuse the collection tube.
6. Adding **600 µL DNA Wash Buffer** diluted with absolute (96%-100%) ethanol. Centrifuge at 10,000 x g for 1 minute and discard the flow-through liquid. Reuse the collection tube in step **7** below.

   Note: DNA Wash Buffer concentrate must be diluted with absolute (96%-100%) ethanol prior to use. Follow directions on label.
7. Repeat wash step with **600 µL DNA Wash Buffer.** Centrifuge at 10,000 x g for 1 minute. Discard flow-through and reuse the collection tube.
8. Centrifuge the empty column for 2 minutes at maximum speed (12, 000 x g) to dry the membrane. This step is ***critical*** for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.
9. Transfer the **DNA Mini Column** to a clean 1.5 mL tube. Apply **100 µL Elution Buffer** (or sterile deionized water) pre-warmed to 65℃ and incubate at room temperature for 3 to 5 minutes. Centrifuge at 10,000 x g for 1 minute to elute DNA. An optional second elution step will yield any residual DNA, though at a lower concentration.
10. Total DNA yields vary depending on type and quantity of sample. Typically, **10-50 µg** DNA with a A_{260}/A_{280} ratio of 1.7-1.9 can be isolated using 50 mg dried tissue.

B. Fresh or Frozen Specimens

Materials to be provided by user:

- Microcentrifuge capable of 10,000 xg.
- Nuclease-free microfuge tubes.

- Waterbath equilibrated to 65℃.
- Equilibrate sterile ddH_2O water at 65℃.
- Absolute (96%-100%) ethanol.
- Liquid nitrogen for freezing/disrupting samples.
- Paper towels.

Note: Use extreme caution when handling liquid nitrogen.

This protocol is suitable for most fresh or frozen tissue samples, allowing efficient recovery of DNA. However, due to the tremendous variation in water and polysaccharide content of various fungi, sample size should be limited to ≦200 mg. 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. Grind the tissue using disposable Kontes pellet pestles. 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 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 carefully wiping the surfaces clean between samples.

1. Collect ground fungal tissue **(**start with **100 mg**) in a microfuge tube and immediately add **600 µL Buffer FG1.** Vortex vigorously. Make sure to disperse all clumps. DNA cannot be effectively extracted from clumped tissue.

   Tip: Process in sets of four to six tubes: fill all tubes with liquid nitrogen, grind, add Buffer FG1, then proceed to step 2 before starting another set. As a starting point, use **100 mg** tissue per tube; if yield and purity are satisfactory increase to **200 mg.**

2. Incubate at 65℃ for 10 minutes. Mix sample twice during incubation by inverting tube several times. Optional: If necessary, add **5 µL** of **RNase A (20 mg/mL)**into the lysate before incubation to remove the RNA.
3. Add **140 µL Buffer FG2** and vortex to mix. Incubate on ice for 5 minutes. Centrifuge at ≧10,000 xg for 10 minutes.

4. Carefully aspirate cleared lysate to a new microfuge tube, making sure not to disturb the pellet or transfer any debris. Add **0.5 Volume Buffer FG3** followed by 1 volume absolute ethanol and vortex to obtain a homogeneous mixture. A precipitate may form upon addition of ethanol (It doesn't interfere with DNA isolation).
5. Apply the entire sample (including any precipitate that may have formed) to a **DNA Mini Column** placed in a **2 mL Collection Tube** (supplied). Centrifuge the column at 10,000 xg for 1 minute to bind DNA. Discard the flow-through liquid.
6. Add **600 µL DNA Wash Buffer** (diluted with absolute ethanol). Centrifuge at 10,000 xg for 1 minute and discard the flow-through liquid. Reuse the collection tube in step 7 below.

   Note: DNA Wash Buffer concentrate must be diluted with absolute (96%-100%) ethanol prior to use. Follow directions on label.
7. Repeat wash step with **600 µL DNA Wash Buffer.** Centrifuge at 10,000 xg for 1 minute. Discard flow-through and reuse the collection tube in step **8**.
8. Centrifuge empty column for 2 minutes at maximum speed to dry the membrane. This step is critical for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.
9. Transfer the **DNA Mini Column** to a clean 1.5 mL tube. Apply **100 µL Elution Buffer** (or sterile deionized water) preheated to 70℃ and incubate at room temperature for 3 to 5 minutes. Centrifuge at 10,000 x g for 1 minute to elute DNA. An optional second elution step will yield any residual DNA, though at a lower concentration.
10. Total DNA yields vary depending on type and quantity of sample. Typically, **20-50 µg DNA** with a A_{260}/A_{280} ratio of 1.7-1.9 can be isolated using **200 mg fresh tissue.**

Vacuum/Spin Protocol

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

1. Prepare wet or dry samples by following the standard protocol in previous sections until loading **DNA/FG3/Ethanol** mixture to a **DNA Mini Column**.
2. Prepare the vacuum manifold according to manufacturer's instructions and connect the **DNA Mini Column** to the manifold.
3. Load the **DNA/FG3/Ethanol solution** to the **DNA Mini 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 turning on the vacuum source. Repeat this step with another **600 µL DNA Wash Buffer**.
6. Assemble the column into the 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** or water. Stand for 1-2 minutes and centrifuge for 1 minute to elute DNA.

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Clogged well	Carry-over of debris.	Following precipitation with Buffer FG2, 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 FG 3 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 Buffer FG1 and FG2 and use two or more columns per sample.
	Incomplete precipitation following addition of FG 2.	Increase RCF or time of centrifugation after addition of Buffer FG2.
Low DNA yield	Incomplete disruption of starting material.	For both dry and fresh samples, obtain a fine homogeneous powder before adding Buffer FG1.
	Poor lysis of tissue.	Decrease amount of starting material or increase amount of Buffer FG1 and FG2.
	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 concentrate 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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