Ver: 1912

Bacterial gDNA Isolation Kit

（BW-GD2411）

Contents

Kit Contents

Catalog#	BW-GD2411 -00	BW-GD2411 -01	BW-GD2411 -02
Preps	4	50	250
DNA Mini Columns	4	50	250
2 mL Collection Tubes	4	50	250
Buffer TL	1.0 mL	13 mL	65 mL
Buffer BL	1.0 mL	13 mL	65 mL
Buffer KB	2.2 mL	28 mL	130 mL
DNA Wash Buffer*	2.0 mL	15 mL	3 x 24 mL
Glass Beads	120 mg	1.5 g	7.0 g
Elution Buffer	2 mL	15 mL	70 mL
Lysozyme**	5 mg	50 mg	5 x 50 mg
Proteinase K	110 µL	1.3 mL	5 x 1.3 mL
RNase A (20 mg/mL)	25 µL	270 µL	1.4 mL
User Manual	1	1	1

*Add 8 mL (BW-GD2411-00) or 60 mL (BW-GD2411-01) or 96 mL (BW-GD2411-02) of absolute ethanol to each bottle.

**Add 100µL (BW-GD2411-00) or 1mL (BW-GD2411-01) or 1mL (BW-GD2411-02) of Elution Buffer to each bottle.

Introduction

The Bacterial gDNA Isolation Kit provides a fast and easy method for isolating gDNA from various sources of bacterial cells. The bacterial cells of logarithmic growth are collected, digested by lysozyme and Proteinase K Following lysis, binding conditions are adjusted and the sample is applied to a DNA column.

Each DNA column can bind approximately 100 µg genomic DNA. Up to 1 x 10^9 bacterial cells

can be processed per column. The kit will isolate all cellular DNA, including plasmid DNA.

Storage and Stability

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

✪ Proteinase K is stable at room temperature (15-25℃) for one year. For long term, store aliquots and lysozyme at -20℃.

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

Before Starting

The kit is designed to be 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

✪ Melt lysozyme to 50 mg/mL according to the standard. It is recommended to pack in several tubes and store at -20℃. Take it out at room temperature before use. Add 100 μL (BW-GD2411-00) or 1 mL (BW-GD2411-01) or 1 mL (BW-GD2411-02) of Elution Buffer to each bottle.

✪ Dilute DNA Wash Buffer with absolute ethanol as follows: Add 8 mL (BW-GD2411-00) or 60 mL (BW-GD2411-01) or 96 mL (BW-GD2411-02) of absolute ethanol to each bottle. The final concentration is 80%.

✪ Under cool ambient conditions, precipitates may form in Buffer BL. In case of such an event, heat the bottle at 37℃ to dissolve before use.

Materials provided by user

- ✪Tabletop centrifuge
- ✪Sterile 1.5 mL centrifuge tubes
- ✪Water bath
- ✪Absolute ethanol

❂Shaking water bath(50℃)

Safety Information

Buffer BL 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.

Bacteria gDNA Isolation Protocols

This method is suitable for the extraction of genomic DNA from 1-3 mL of bacterial culture in logarithmic growth phase.

For Gram-Negative Bacteria

1. Take 1-3 mL of bacterial culture solution and centrifuge at 12,000 x g for 2 minutes. Discard the supernatant. Resuspend the pellet with 200 µL Buffer TL and **25 µL Proteinase K**. **Optional:** Add **5 µL** of **RNase A** (20 mg/mL) to the sample and mix well by pipetting.
2. Add **220 µL Buffer BL**, vortex, and incubate at 55℃ for 10 minutes. Fibrous pellets may form after addition of the BL buffer (precipitation does not affect DNA purification).
3. Add 220 µL of absolute ethanol and vortex for 20 seconds to mix.
4. Insert the **DNA Mini Column** into a collection tube. Transfer the sample from step **3** to the column. Centrifuge at 10,000 x g for 1 minute to bind the DNA. Discard the flow-through liquid.
5. Put the column back into the collection tube and add **500 µL Buffer KB**. Centrifuge at 10,000 x g for 1 minute. Discard the flow-through liquid and put the column back into the collection tube.
6. Add **600 µL** of **DNA Wash Buffer** (Add absolute ethanol before use). Centrifuge at 10,000 x g for 1 minute. Discard flow-through liquid and put the column back to the collection tube
7. Repeat the step **6**.

8. Put the empty column, **with the lid open**, into the same collection tube and centrifuge at 12,000 x 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. Put the column into a clean centrifuge tube, and add **50-200 µL** of **Elution Buffer** to the center of the membrane. Allow it stand at room temperature for 2 minutes. Centrifuge at 12,000 x 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.\

Bacteria gDNA Isolation Protocols

For Gram-Positive Bacteria

1. Take 1-3 mL of bacterial culture solution and centrifuge at 12,000 x g for 2 minutes. Discard the supernatant.

2. Resuspend the pellet with **180 µL** of TE or **Elution Buffer**. Add **18 µL lysozyme** and **5 µL** of **RNase A** (20 mg/mL), and incubate at 30℃ for 30 minutes.

   Note: Complete digestion of the cell wall is essential for efficient lysis. Longer incubation time may yield more genomic DNA.

3. Centrifuge at 5,000 x g for 5 minutes, discard the flow-through liquid and reserve **10 µL** of supernatant.

4. Add a few glass beads (approximately 25 mg) and **200 µL** of **Buffer TL**,resuspend the cells, vortex for 2 minutes at maximum speed. Allow the glass beads to settle at the bottom of the tube. Transfer the supernatant to a 1.5 mL centrifuge tube.

5. Add **25 µL** of **Proteinase K**, vortex for 10 seconds, and centrifuge briefly.

6. Put the mixture in a 50℃ shaking incubator for 30 minutes. If there is no shaking incubator, vortex once every 2-3 minutes to mix.

7. Add **220 µL Buffer BL**, vortex, and incubate at 65℃ for 10 minutes. Fibrous pellets may form after addition of the **Buffer BL** (precipitation does not affect DNA purification).

8. Add 220 µL of absolute ethanol and vortex 20 seconds to mix well.

9. Insert a **DNA Mini Column** into a **2 mL Collection Tube**, transfer all the mixture in step **8** to the column, and centrifuge at 10,000 x g for 1 minute. Discard the flow-through liquid.

10. Put the column back into the collection tube and add **500 µL Buffer KB**. Centrifuge at 10,000 x g for 1 minute. Discard flow-through liquid and put the column back to the collection tube

11. Add **600 µL** of **DNA Wash Buffer** (Add absolute ethanol before use). Centrifuge at 10,000 x g for 1 minute. Discard flow-through liquid and put the column back to the collection tube.

12. Repeat the step **11**.

13. Put the empty column, with the lid open, into the same collection tube and centrifuge at 12,000 x 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.

14. Transfer the column into a clean centrifuge tube. Add **50-200 µL** of **Elution Buffer** to the center of the membrane. Allow it stands at room temperature for 2 minutes. Centrifuge at 12,000 x 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) x (0.05 µg/µL) x (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.

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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