

ODP302

Bacteria DNA Kit

For extraction of genomic DNA from Bacteria

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Spin Column

Kit Contents

Contents	50 Preps
Buffer GA	10mL
Buffer GB	10mL
Buffer AB	5mL
Buffer GD	13mL
Buffer PW	15mL
Buffer TE	10mL
Proteinase K(20mg/mL)	1mL
Spin column CB3 with Collection Tubes (2mL)	50

Storage

Bacteria DNA kit could be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer storage, the kit could be stored at 2-8°C.

(Note: Check buffers for precipitate before use and dissolve at 37°C for 10 minutes if necessary)

Proteinase K should be stored at -20°C.

Lysozyme should be stored in aliquots to prevent repeated freeze-thaw cycles.

Introduction

Bacteria DNA Kit is based on silica membrane technology and special buffer system for genomic DNA extraction. The spin column made of new type silica membrane, that can bind DNA optimally on given salt and pH conditions. Simple centrifugation processing completely removes contaminants and enzyme inhibitors such as proteins and divalent cations. Extracted DNA is eluted in low-salt buffer or water, ready for use in downstream applications.

DNA extracted by Bacteria DNA Kit is suited for restriction analysis, PCR analysis, Southern blotting, and cDNA library.

Important Notes

1. Add appropriate amount of ethanol (96-100%) to Buffer GD and Buffer PW as indicated on the bottle before use.
Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.
2. All centrifugation steps are carried out at 13,400 ×g (12,000 rpm) in conventional tabletop microcentrifuge at room temperature.
3. Increasing the time of absorption and elution could improve recovery efficiency.
4. If a precipitate has formed in Buffer GB or Buffer GD, warm the buffer to 56°C until the precipitate has fully dissolved.

Protocol

- **Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96-100%) as indicated on the bottle and shake thoroughly.**
 - **Add 100µL Buffer AB to the center of spin column, keep at room temperature for 5 minutes and then centrifuge at 12000 rpm for 2 minutes, before using the spin column.**
1. Pipette 1-5mL bacterial culture suspension in a microcentrifuge tube by centrifuging for 1 minute at 12,000 rpm (~13,400 ×g). Discard supernatant.
 2. Add 180µL Buffer GA. Mix thoroughly by vortexing.
Note: For the more difficult Gram-positive bacteria, you can skip Step 2, and use Lysozyme (50mg/mL) for lysis.
 - **Add 180µL enzymatic lysis buffer to the bacterial pellet, followed by 20µL Lysozyme.**
Note: Lysozyme should be stored in aliquots to prevent repeated freeze-thaw cycles.
 - **Enzymatic lysis buffer composition:**
 - 20mM Tris·Cl, pH 8.0
 - 2mM sodium EDTA
 - 1.2% Triton X-100
 - **Incubate for at least 30 minutes at 37°C.**
 - **If RNA-free genomic DNA is required, add 4µL RNase A (10mg/mL) (Cat. No.: ORT405-01, Not supplied), mix by vortexing for 15 seconds, and incubate for 5 minutes at room temperature (15–25°C).**
 3. Add 20µL Proteinase K, mix thoroughly by vortexing.
 4. Heat the sample at 56°C for 10 minutes to yield a homogeneous solution.
 5. Add 200µL Buffer GB to the sample, mix thoroughly by vortexing,
Optional: Incubate at 70°C for 10 minutes to yield a homogeneous solution.
Note: Precipitates are expected, but it will not interfere with the extraction.
 6. Add 200µL ethanol (96-100%) to the sample, and mix thoroughly by vortexing for 20 seconds. Briefly centrifuge the 1.5mL microcentrifuge tube at 1,000 - 3,000 rpm for 30 seconds to remove drops from the inside of the lid.
Note: Precipitate formed in the earlier step will be dissolved.

Before transferring the lysate to spin column, add 100µL Buffer AB to the center of Spin column, keep at room temperature for 5 minutes and then centrifuge at 12000 rpm for 2 minutes.

7. Pipette the mixture from step 6 into the spin column CB3 (in a 2mL collection tube) and incubate at room temperature for 5-10 minutes. Centrifuge at 12,000 rpm (~13,400 ×g) for 45 seconds and discard flow-through. Place the spin column CB3 into the collection tube.
8. Add 500µL Buffer GD to spin column CB3, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds, then discard the flow-through and place the spin column into the collection tube.

Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.

9. Add 700µL Buffer PW to spin column CB3, and centrifuge at 12,000 rpm (~13,400 ×g) for 30 seconds. Discard the flow-through and place the spin column into the collection tube.
10. Repeat step 9.
11. Centrifuge for 2 minutes at 12,000 rpm (~13,400 ×g) to remove residual wash Buffer PW. Discard the collection tube and transfer the spin column CB3 to a clean 1.5mL or 2mL microcentrifuge tube. Open the lid of the spin column CB3 and incubate the assembly at room temperature (15-25°C) for 2 minutes to dry membrane completely.

Note: Residual ethanol from Buffer PW may inhibit subsequent enzymatic reactions.

12. Pipette 50–200µL Buffer TE directly onto the membrane, incubate for 2-5 minutes at room temperature (15–25°C), and then centrifuge for 2 minutes at 12,000 rpm (~13,400 ×g) to elute.

Note: To increase the DNA yield,

- Introduce the eluted Buffer TE to the spin column CB3 and centrifuge for 2 minutes at 12,000 rpm.
- Warm Buffer TE at 50-60°C before adding to the spin column CB3.

If the volume of eluted buffer is less than 50µL, it may affect recovery efficiency. What's more, the pH value of eluted buffer will have some influence in eluting; we suggest Buffer TE or distilled water (pH 7.0 - 8.5) to elute genomic DNA. For long-term storage of DNA, eluting in Buffer TE and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.