

Bacteria Genomic DNA Kit

Cat. No.: B1522827 | Pack size: 50T | Storage: Room temperature

Overview

This kit is suitable for rapid and simple extraction of genomic DNA from bacterial samples. The extracted genomic DNA has large fragments, high purity, and good stability, and can be directly used in experiments such as PCR, restriction enzyme digestion, and nucleic acid hybridization. The extraction process does not require phenol-chloroform extraction. Bacterial samples are lysed with a lysis buffer and digested with proteinase K. Then, in the presence of absolute ethanol, the genomic DNA binds specifically to the WHF spin column. After rapid and thorough washing steps, residual proteins, salts, and other impurities are effectively removed. Finally, the DNA is dissolved in Buffer EB, yielding high-purity genomic DNA.

Kit Contents & Storage

Product No.	Component	Appearance	50T	Storage
B1522827A	Buffer UA	Liquid	15 mL	RT
B1522827B	Buffer UL	Liquid	15 mL	RT
B1522827C	Buffer PW1	Liquid	17 mL	RT
B1522827D	Buffer PW2	Liquid	15 mL	RT
B1522827E	Buffer EB	Liquid	15 mL	RT
B1522827F	Lysozyme Digestion Buffer	Liquid	9 mL	RT
B1522827G	Proteinase K	Liquid	1.2 mL	RT
B1522827H	WHF Spin Column (with 2 mL collection tube)	-	1 EA × 50	RT

All components of this kit can be stored under dry conditions at room temperature for 15 months.

Precautions

Samples should be protected from repeated freeze-thaw cycles, which may result in smaller DNA fragments and reduced yield.

If RNA removal is required, the user needs to supply RNase A solution (100 mg/mL). Refer to the relevant procedure step for usage.

The user needs to supply absolute ethanol for pretreatment of Buffer PW1 and Buffer PW2. For Gram-positive bacteria extraction, lysozyme solution (50 mg/mL) (Cat. No. L1520264) is also required.

Before use, check Buffer UA for crystallization or precipitation. If present, redissolve by placing in a 37°C water bath.

Procedure

Before use, add 22 mL of absolute ethanol to Buffer PW1 and 60 mL of absolute ethanol to Buffer PW2.

I. Genomic DNA Extraction from Gram-Negative Bacteria

1. Transfer 1-5 mL of bacterial culture (containing 10^6 - 10^9 cells, maximum 2×10^9 cells) to a user-supplied centrifuge tube. Centrifuge at 12,000 rpm ($13,680 \times g$) for 1 min. Remove the supernatant completely, retaining the bacterial pellet.

2. Add 200 μ L of Buffer UA to the bacterial pellet and vortex until the cells are completely resuspended.

3. Add 20 μ L of Proteinase K, vortex to mix, and incubate at 56°C until the solution becomes clear. During incubation, invert or vortex the tube periodically to disperse the sample and promote lysis.

[Note] If RNA removal is required, after the above step add 4 μ L of RNase A solution (100 mg/mL), vortex for 15 sec, and let stand at room temperature for 15-20 min.

4. Add 200 μ L of Buffer UL and vortex thoroughly to mix. Then add 200 μ L of absolute ethanol and vortex thoroughly to mix. Briefly centrifuge to collect the solution from the cap wall, avoiding reagent residue.

[Notes]

(1) If extracting multiple samples simultaneously, Buffer UL and absolute ethanol can be premixed in equal proportions, and 400 μ L of the mixture can be added to each tube and vortexed thoroughly to improve efficiency.

(2) White precipitate may appear after adding Buffer UL and absolute ethanol; this is normal and does not affect subsequent steps.

5. Transfer the solution and precipitate from step 4 into a WHF spin column (placed in the provided 2 mL collection tube). Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 sec. Discard the flow-through and return the spin column to the collection tube.

6. Add 700 μ L of Buffer PW1 (with ethanol pre-added) to the spin column. Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 sec. Discard the flow-through and return the spin column to the collection tube.

7. Add 700 μL of Buffer PW2 (with ethanol pre-added) to the spin column. Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 sec. Discard the flow-through and return the spin column to the collection tube.

[Note] To further increase DNA purity, repeat step 7.

8. Centrifuge the spin column together with the collection tube at 12,000 rpm ($13,680 \times g$) for 2 min to completely remove residual wash buffer and impurities (this avoids residual ethanol affecting subsequent DNA elution and experiments).

9. DNA elution

Transfer the spin column to a new 1.5 mL centrifuge tube. Open the cap and let stand for 2 min to allow residual ethanol to evaporate completely. Slowly add 50-200 μL of Buffer EB dropwise to the center of the membrane without touching it. Let stand at room temperature for 1 min. Centrifuge at 12,000 rpm ($13,680 \times g$) for 30 sec. Collect the DNA solution in the centrifuge tube. The DNA can be used immediately for downstream experiments or stored at -20°C for long-term preservation.

[Notes]

(1) If downstream applications are sensitive to pH or EDTA, sterile water can be used for elution. The pH of the elution buffer significantly affects elution efficiency. If water is used, ensure its pH is between 7.0 and 8.5 (adjust with NaOH). pH values below 7.0 will reduce elution efficiency.

(2) To increase the final DNA concentration, reload the collected DNA eluate from step 9 onto the same membrane, let stand at room temperature for 2 min, centrifuge at 12,000 rpm ($13,680 \times g$) for 30 sec.

(3) For long-term storage, it is recommended to elute with Buffer EB and store at -20°C to effectively prevent DNA degradation.

II. Genomic DNA Extraction from Gram-Positive Bacteria

1. Transfer 1-5 mL of bacterial culture (containing 10^6 - 10^9 cells, maximum 2×10^9 cells) to a user-supplied centrifuge tube. Centrifuge at 12,000 rpm ($13,680 \times g$) for 1 min. Remove the supernatant completely, retaining the bacterial pellet.

2. Add 160 μL of Lysozyme Digestion Buffer and 40 μL of lysozyme solution (50 mg/mL) (user-supplied) to the bacterial pellet. Vortex until the cells are completely resuspended with no visible pellet.

3. Incubate the tube at 37°C for 30 min to thoroughly disrupt the bacterial cell wall.

4. Add 20 μL of Proteinase K and vortex for 10 sec. Then add 200 μL of Buffer UL and vortex thoroughly to mix.

[Note] Do not add Proteinase K directly into Buffer UL; add in the order specified.

5. Incubate the tube at 56°C for 30 min to further promote cell lysis and protein digestion.

【Note】 If RNA removal is required, after the above step add 4 μL of RNase A solution (100 mg/mL), vortex for 15 sec, and let stand at room temperature for 15-20 min.

6. Add 200 μL of absolute ethanol and vortex thoroughly to mix. Briefly centrifuge to collect the solution from the cap wall, avoiding reagent residue.

【Note】 White precipitate may appear after adding absolute ethanol; this is normal and does not affect subsequent steps.

7. Transfer the solution and precipitate from step 6 into a WHF spin column (placed in the provided 2 mL collection tube). Centrifuge at 12,000 rpm (13,680 $\times$ g) for 30 sec. Discard the flow-through and return the spin column to the collection tube.

8. Add 700 μL of Buffer PW1 (with ethanol pre-added) to the spin column. Centrifuge at 12,000 rpm (13,680 $\times$ g) for 30 sec. Discard the flow-through and return the spin column to the collection tube.

9. Add 700 μL of Buffer PW2 (with ethanol pre-added) to the spin column. Centrifuge at 12,000 rpm (13,680 $\times$ g) for 30 sec. Discard the flow-through and return the spin column to the collection tube.

【Note】 To further increase DNA purity, repeat step 9.

10. Centrifuge the spin column together with the collection tube at 12,000 rpm (13,680 $\times$ g) for 2 min to completely remove residual wash buffer and impurities (this avoids residual ethanol affecting subsequent DNA elution and experiments).

11. DNA elution

Transfer the spin column to a new 1.5 mL centrifuge tube. Open the cap and let stand for 2 min to allow residual ethanol to evaporate completely. Slowly add 50-200 μL of Buffer EB dropwise to the center of the membrane without touching it. Let stand at room temperature for 1 min. Centrifuge at 12,000 rpm (13,680 $\times$ g) for 30 sec. Collect the DNA solution in the centrifuge tube. The DNA can be used immediately or stored at -20°C for long-term preservation.

【Notes】

(1) If downstream applications are sensitive to pH or EDTA, sterile water can be used for elution. The pH of the elution buffer significantly affects elution efficiency. If water is used, ensure its pH is between 7.0 and 8.5 (adjust with NaOH). pH values below 7.0 will reduce elution efficiency.

(2) To increase the final DNA concentration, reload the collected DNA eluate from step 11 onto the same membrane, let stand at room temperature for 2 min, centrifuge at 12,000 rpm (13,680 $\times$ g) for 30 sec.

(3) For long-term storage, it is recommended to elute with Buffer EB and store at -20°C to effectively prevent DNA degradation.

DNA Concentration and Purity Assessment

The fragment size of the extracted genomic DNA is influenced by factors such as sample storage time and shear forces during handling. The purified DNA can be assessed for concentration and purity by agarose gel electrophoresis and UV spectrophotometry.

1. Concentration determination

Measure the absorbance of the sample at 260 nm (A_{260}) using a UV spectrophotometer to determine DNA concentration. If dilution is required, Buffer EB is recommended. Dilution with water may lead to inaccurate readings. The absorbance at 260 nm should be kept within 0.1-1.0 to ensure accuracy. Conversion: 1 unit of absorbance at 260 nm corresponds to 50 µg/mL of double-stranded DNA or 40 µg/mL of single-stranded DNA.

2. Purity assessment

Evaluate DNA purity by the A_{260}/A_{280} ratio. For high-quality genomic DNA, the A_{260}/A_{280} ratio should be between 1.7 and 1.9. If ddH₂O instead of Buffer EB is used for elution, the ratio may be lower due to the influence of pH and ions on light absorption; this does not necessarily indicate low DNA purity.

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