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This product is for scientific research only, which shall not be used for clinical diagnosis or other purposes.

Bacteria Genomic DNA Kit

Cat. No. : CW0552S (50 preps)

Storage Condition : Store at room temperature (15-30°C)

Components

Component	CW0552S (50 preps)
	50 preps
Buffer GTL	15 mL
Buffer GL	15 mL
Buffer GW1 (concentrate)	13 mL
Buffer GW2 (concentrate)	15 mL
Buffer GE	15 mL
Proteinase K	1.25 mL
Spin Columns DM with Collection Tubes	50

Introduction

This kit is suitable for extracting high purity total DNA from Gram-negative and Gram-positive bacteria. It can target 106~108 cells at a time and obtain up to 20 μg of total DNA. In the purification process, no toxic solvents such as phenol or chloroform, no ethanol precipitation, high purity DNA can be obtained within one hour. This kit uses an optimized buffer system to bind the DNA in the lysate to the silicon matrix centrifugal adsorption column efficiently and specifically, while other contaminants can flow through the membrane. PCR and other inhibitors of enzymatic reactions can be effectively removed through a two-step washing process. Finally, low-salt buffer or water removal is used to obtain high purity DNA. The purified DNA can be directly used in enzyme digestion, PCR, Real-Time PCR, library construction, Southern Blot, molecular labeling and other downstream experiments.

Reagents to be Supplied by user

100% ethanol

Enzymatic Lysis Buffer is required for extracting Gram-positive bacteria.

Enzymatic Lysis Buffer preparation method: 20 mM Tris, pH8.0; 2 mM Na_2-EDTA, pH8.0; 1.2% Triton X-100. After sterilization at 121℃ for 20 min, the final concentration of Lysozyme is 20 mg/mL. The detailed preparation method can be logged on the website of CWBIO and searched for Cat. No.: CW0552S products.

2.9 Add 500 μL Buffer GW2 to the adsorption column (check whether 100% ethanol has been added before use), centrifuge at 12,000 rpm for 1 min, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

Note:

Repeat Step 9 to further improve DNA purity.

2.10 Centrifuge at 12,000 rpm for 2 min and discard the waste liquid in the collection tube. Place the adsorption column at room temperature for a few minutes to dry thoroughly.

Note:

The purpose of this step is to remove the residual ethanol in the adsorption column, which will affect the subsequent enzymatic reaction (enzyme digestion, PCR, etc.).

2.11 The adsorption column is placed in a new centrifuge tube (self-provided), and 50-200 μL Buffer GE is added to the middle part of the adsorption column. The adsorption column is placed at room temperature for 2-5 min, centrifuged at 12,000 rpm for 1 min, and DNA solution is collected and stored at -20°C.

Note:

1) If the downstream experiment is sensitive to pH or EDTA, it can be eluted with RNase-Free water. The pH value of the eluent has a great influence on the elution efficiency. If water is used as the eluent, the pH value should be within 7.0~8.5 (NaOH can be used to adjust the pH value of water to this range). When pH value is lower than 7.0, the elution efficiency is not high.

2) Incubation at room temperature for 5 min before centrifugation can increase yield.

3) Eluting with another 50-200 μL Buffer GE or RNase-Free water can increase the yield.

4) If the final concentration of DNA is to be increased, the DNA eluent obtained in step 11 can be re-added to the adsorption membrane and Step 11 can be repeated; If the elution volume is less than 200 μL, the final DNA concentration can be increased, but the total production may be reduced. If the amount of DNA is less than 1 μg, eluting with 50 μL Buffer GE or RNase-Free water is recommended.

5) DNA stored in water will be affected by acid hydrolysis. If long-term preservation is required, Buffer GE is recommended for elution and storage at -20°C.

2. The extraction of genomic DNA of Gram-positive bacterium

2.1 1-5 mL of bacterial culture (10^6-10^8 cells, no more than 2×10^9 cells) is placed in a centrifuge tube (self-provided), centrifuged at 12,000 rpm (~13,400×g) for 1 min, and the supernatant is absorbed as much as possible.

2.2 The 180 μL Enzymatic Lysis Buffer is added to resuspend bacterial precipitation. Enzymatic Lysis Buffer is prepared from self-contained reagents in the section before the instructions.

2.3 Incubate at 37°C for 30 min.

2.4 Add 20 μL Proteinase K vortex and mix thoroughly. Add 200 μL Buffer GL, swirl and mix well.

Note:

Do not add Proteinase K directly to Buffer GL.

2.5 Incubate at 56°C for 30 min.

Note:

1) Incubation at 95°C for 15 minutes inactivates the pathogen if needed, but 95°C incubation causes some DNA degradation.

2) To remove RNA, add 4 μL of 100 mg/mL RNase A solution (Cat. No.: CW0601S) after the above steps are completed, shake and mix, and place at room temperature for 5-10 min.

2.6 Add 200 μL 100% ethanol and mix thoroughly in vortex.

Note:

Adding 100% ethanol may produce white precipitation, which will not affect the subsequent experiment.

2.7 Add all the solution obtained in step 6 (including the precipitates formed) into Spin Columns DM which has been loaded into the collection tube. If the solution cannot be added at one time, it can be transferred into the spin columns for several times. Centrifuge at 12,000 rpm for 1 min, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

2.8 Add 500 μL Buffer GW1 to the adsorption column (check whether 100% ethanol has been added before use), centrifuge at 12,000 rpm for 1 min, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

Precautions

1. The sample should avoid repeated freezing and thawing, otherwise the extracted DNA fragments will be smaller and the extracted amount will decrease.
2. If extracting the genome of a bacterial culture with large accumulation of secondary metabolites or thick cell walls, it is recommended to collect samples early in the logarithmic growth phase.
3. 100% ethanol should be added to Buffer GW1 and Buffer GW2 according to reagent bottle label instructions prior to first use.
4. Please check whether Buffer GTL and Buffer GL are crystallized or precipitated before use. If any crystallization or precipitation is found, please re-dissolve Buffer GL and Buffer GTL in water bath at 56°C.
5. If the downstream test is sensitive to RNA contamination, you can add 4 μL DNase-Free RNase A (100 mg/mL) before adding Buffer GL. RNase A is not provided in this kit. If necessary, you can order RNase A separately from our company (Cat. No.: CW0601S).
6. If the sample is Gram-positive, the Enzymatic Lysis Buffer is prepared by the customer and uses the 20 mg/mL Lysozyme that is not provided in this kit. Enzymatic sozyme can be purchased from us separately (Cat. No.: CW0887S).

Protocol

1. Extraction of genomic DNA of Gram-negative bacteria

1.1 Take 1-5 mL of bacterial culture (10^6-10^8 cells, no more than 2×10^9 cells) and place it in a centrifuge tube (prepared by yourself). Centrifuge at 12,000 rpm (~13,400×g) for 1 min, and try to absorb the upper clear as much as possible.

1.2 180 μL Buffer GTL was added to the precipitate, and the weight of bacteria is suspended by oscillation.

1.3 Add 20 μL Proteinase K, vortex mix, and incubate at 56°C until the solution becomes clear. During incubation, reverse or shake the centrifuge tube at intervals to disperse the samples.

Note:

To remove RNA, add 4 μL of 100 mg/mL RNase A solution (Cat. No.: CW0601S) after the above steps, shake and mix, and leave at room temperature for 5~10 min.

1.4 Add 200 μL Buffer GL and mix thoroughly with vortex shock. Add 200 μL 100% ethanol and mix thoroughly in vortex. Centrifuge briefly to collect the solution from the wall to the bottom of the tube.

Note:

1) If multiple samples are used together, Buffer GL and 100% ethanol can be mixed in equal proportions and then added together for shock mixing.

2) White precipitation may be produced after the addition of Buffer GL and 100% ethanol, which will not affect the subsequent experiment.

1.5 Add all the solution obtained in step 4 (including the precipitates formed) into Spin Columns DM which has been loaded into the collection tube. If the solution cannot be added all at once, it can be transferred into the spin columns for several times. Centrifuge at 12,000 rpm for 1 min, discard the waste liquid, and put the adsorption column back into the collection tube.

1.6 Add 500 μL Buffer GW1 to the adsorption column (check whether 100% ethanol has been added before use), centrifuge at 12,000 rpm for 1 min, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

1.7 Add 500 μL Buffer GW2 to the adsorption column (check whether 100% ethanol has been added before use), centrifuge at 12,000 rpm for 1 min, discard the waste liquid in the collection tube, and put the adsorption column back into the collection tube.

Note:

Repeat Step 7 if you need to further improve DNA purity.

1.8 Centrifuge at 12,000 rpm for 2 minutes and discard the waste liquid in the collection tube. Leave the adsorption column at room temperature for a few minutes to dry thoroughly.

Note: The purpose of this step is to remove residual ethanol from the adsorption column, which will affect subsequent enzymatic reactions (enzyme digestion, PCR, etc.).

1.9 The adsorption column is placed in a new centrifugal tube, and 50-200 μL Buffer GE is added to the middle part of the adsorption column. The adsorption column is placed at room temperature for 2-5 min and centrifuged at 12,000 rpm for 1 min. DNA solution was collected and stored at -20°C.

Note:

1) If the downstream experiment is sensitive to pH or EDTA, it can be eluted with RNase-Free water. The pH value of the eluent has a great influence on the elution efficiency. If water is used as the eluent, ensure that the pH value is within 7.0~8.5 (NaOH can be used to adjust the pH value of water to this range). When pH value is lower than 7.0, the elution efficiency is not high.

2) Incubation at room temperature for 5 min before centrifugation can increase yield.

3) Eluting with another 50~200 μL Buffer GE or sterilized water can increase the yield.

4) If the final concentration of DNA is to be increased, the DNA eluent obtained in Step 9 can be re-added to the adsorption membrane and Step 9 can be repeated; If the elution volume is less than 200 μL, the final DNA concentration can be increased, but the total production may be reduced. If the amount of DNA is less than 1 μg, eluting with 50 μL Buffer GE or sterilized water is recommended.

5) DNA stored in water will be affected by acid hydrolysis. If long-term preservation is required, Buffer GE is recommended for elution and storage at -20°C.