Spin Bacterial DNA Kit

Cat.No.: HYC263

Product Description

This kit is suitable for extracting high-purity total DNA from Gram-negative and Gram-positive bacteria. It can process 10^6-10^9 cells per operation and yield up to 20 μg of total DNA. No toxic solvents such as phenol or chloroform are required during purification, and high-purity DNA can be obtained within one hour. Adopting an optimized buffer system, this kit enables efficient and specific binding of DNA in lysate to silica-membrane spin columns, while other contaminants flow through the membrane. Inhibitors of PCR and other enzymatic reactions are effectively removed via two washing steps. Finally, high-purity DNA is eluted with low-salt buffer or pure water. The purified DNA can be directly applied to downstream experiments including enzyme digestion, PCR, Real-Time PCR, library construction, Southern Blotting and molecular marker analysis.

Components

Components	50T
Buffer ATL	15 mL
Buffer AL	15 mL
Buffer AW1*	13 mL
Buffer AW2*	15 mL
Buffer EB	15 mL
Proteinase K	1.25 mL
Spin Columns DM with Collection Tubes	50

Storage

All components can be stably stored in a dry place at room temperature (15-30℃).

Required Reagents and Equipment

- Anhydrous Ethanol
- Enzymatic Lysis Buffer shall be prepared by users for extraction of Gram-positive bacteria.

- Preparation of Enzymatic Lysis Buffer: 50 mM Tris (pH 8.0), 10 mM Na_2-EDTA (pH 8.0). Sterilize at 121 °C for 20 minutes.
- Preparation of Lysozyme Stock Solution: Add appropriate amount of lysozyme into sterilized Enzymatic Lysis Buffer to reach a final concentration of 100 mg/mL.
  Example: Add 100 mg lysozyme into 1 mL Enzymatic Lysis Buffer to obtain a final concentration of 100 mg/mL.

Instructions

Preparations

- Avoid repeated freeze-thaw cycles of samples, otherwise the extracted DNA will be fragmented and the yield will decrease.
- For genomic DNA extraction from bacterial cultures with abundant secondary metabolites or thick cell walls, collect samples at the early logarithmic growth phase.
- Prior to first use, add anhydrous ethanol to Buffer AW1 and Buffer AW2 in accordance with the instructions on reagent bottle labels.
- Check Buffer ATL and Buffer AL for crystals or precipitates before use. If any appear, dissolve them completely by water bath incubation at 56 °C.
- If extracting samples of Gram-positive bacteria, users need to prepare Enzymatic Lysis Buffer independently for bacterial cell treatment.

Extraction of Genomic DNA from Gram-negative Bacteria

1. Transfer 1–5 mL bacterial culture (10^6–10^9 cells, maximum no more than 2×10^9 cells) into a self-provided centrifuge tube. Centrifuge at 12,000 rpm (~13,400×g) for 1 min, and remove the supernatant completely.
2. Add 200 µL Buffer ATL to the pellet, and vortex thoroughly to resuspend the bacterial cells.
3. Add 20 µL Proteinase K, mix well by vortexing, then incubate at 56 °C until the solution becomes clear. Invert or shake the tube periodically during incubation to disperse the sample evenly.
4. Add 200 µL Buffer AL and vortex thoroughly to mix well. Then add 200 µL anhydrous ethanol and mix evenly by vortexing. Perform brief centrifugation to collect all liquid droplets on the tube wall to the bottom of the tube.

1) For batch processing of multiple samples, pre-mix Buffer AL and anhydrous ethanol in equal proportion before adding together and mixing thoroughly.

2) *White precipitate may form after adding Buffer AL and anhydrous ethanol, which will not interfere with subsequent experiments.*

5. Transfer all liquid obtained in Step 4 (including precipitate) into Spin Columns DM assembled in a collection tube. Add in several portions if needed. Centrifuge at 10,000 rpm for 1 min, discard the waste liquid, and place the spin column back into the collection tube.
6. Add 500 µL Buffer AW1 (confirm anhydrous ethanol has been added before use) into the spin column. Centrifuge at 13,000 rpm for 1 min, discard waste liquid and reassemble the column.
7. Add 500 µL Buffer AW2 (confirm anhydrous ethanol has been added before use) into the spin column. Centrifuge at 13,000 rpm for 1 min, discard waste liquid and reassemble the column.

   Repeat Step 7 for higher DNA purity if required.
8. Centrifuge at 13,000 rpm for 2 min and discard waste liquid. Place the spin column at room temperature for several minutes to thoroughly dry residual ethanol.

   This step is to remove residual ethanol in the column, as ethanol residue will inhibit downstream enzymatic reactions such as enzyme digestion and PCR.
9. Place the spin column into a new centrifuge tube. Pipette 50–200 µL Buffer EB vertically onto the center of the column membrane without touching it. Let stand at room temperature for 2–5 min, then centrifuge at 10,000 rpm for 1 min to elute DNA. Store eluted DNA at -20 °C.

   1) Use sterile water for elution if downstream experiments are sensitive to pH value or EDTA. Elution efficiency is greatly affected by pH. Ensure the pH of elution water is within 7.0–8.5 (adjust with NaOH if necessary); low pH below 7.0 will reduce elution efficiency.

   2) Incubate for 5 minutes at room temperature before centrifugation to increase DNA yield.

   3) Re-elute with another 50–200 µL Buffer EB or sterile water to obtain higher yield.

   4) To increase final DNA concentration, reload the eluate onto the column membrane and repeat Step 9. Elution volume less than 200 µL raises DNA concentration yet may lower total yield. Elute with 50 µL Buffer EB or sterile water if total DNA yield is less than 1 µg.

5) DNA stored in water is susceptible to acid hydrolysis. For long-term storage, elute with Buffer EB and preserve at -20 °C.

Extraction of Genomic DNA from Gram-positive Bacteria

1. Transfer 1–5 mL bacterial culture (10^6–10^9 cells, maximum no more than 2×10^9 cells) into a self-supplied centrifuge tube. Centrifuge at 12,000 rpm (~13,400×g) for 1 minute and remove the supernatant completely.
2. Add 160 µL Enzymatic Lysis Buffer and 20 µL self-prepared 100 mg/mL Lysozyme solution to resuspend the bacterial pellet. Refer to the self-prepared reagents section above for Enzymatic Lysis Buffer preparation method.
3. Incubate the mixture at 37 °C for 30 minutes.
4. Add 20 µL Proteinase K and mix thoroughly by vortexing, then add 200 µL Buffer AL and mix well.
   Do not add Proteinase K directly into Buffer AL.
5. Incubate at 56 °C for 30 minutes.
   If necessary, incubate at 95 °C for 15 minutes to inactivate pathogens; however, this treatment may cause partial DNA degradation.
6. Add 200 µL anhydrous ethanol and vortex vigorously to mix evenly.
   White precipitate may appear after adding anhydrous ethanol, which has no adverse effect on subsequent experiments.
7. Transfer all solution obtained in Step 6 including precipitates into Spin Columns DM placed in a collection tube. Transfer in portions if needed. Centrifuge at 10,000 rpm for 1 minute, discard waste liquid and put the spin column back into the collection tube.
8. Add 500 µL Buffer AW1 (confirm anhydrous ethanol is added in advance) into the spin column. Centrifuge at 13,000 rpm for 1 minute, discard waste liquid and reassemble the column.
9. Add 500 µL Buffer AW2 (confirm anhydrous ethanol is added in advance) into the spin column. Centrifuge at 13,000 rpm for 1 minute, discard waste liquid and reassemble the column.
   Repeat Step 9 to obtain higher-purity DNA.

10. Centrifuge at 13,000 rpm for 2 minutes and discard waste liquid. Place the spin column at room temperature for several minutes to dry completely.
    This step removes residual ethanol inside the column, as residual ethanol will interfere with downstream enzymatic reactions such as enzyme digestion and PCR.
11. Place the spin column into a new self-supplied centrifuge tube. Pipette 50–200 μL Buffer EB vertically onto the center of the column membrane without contact. Keep at room temperature for 2–5 minutes, then centrifuge at 10,000 rpm for 1 minute to collect DNA eluate. Store DNA sample at -20 °C.
    1) Use sterile water for elution if downstream experiments are sensitive to pH value or EDTA. Elution efficiency is highly dependent on pH. Ensure the pH of elution water ranges from 7.0 to 8.5 (adjust with NaOH if needed); elution efficiency will decrease obviously when pH is lower than 7.0.
    2) Extend room-temperature incubation to 5 minutes before centrifugation to increase DNA yield.
    3) Perform second elution with additional 50–200 μL Buffer EB or sterile water to obtain more DNA.
    4) To raise final DNA concentration, reload the eluted DNA solution onto the silica membrane and repeat Step 11. Elution volume less than 200 μL increases DNA concentration but reduces total yield. It is recommended to elute with 50 μL Buffer EB or sterile water when total DNA content is less than 1 μg.
    5) DNA preserved in pure water is prone to acid hydrolysis. For long-term storage, elute DNA with Buffer EB and store at -20 °C.