Tribioscience

Column-Pure Bacterial Genomic DNA Isolation Kit

Catalog	Unit
TBS6101	50 Preparations

Description

Column-Pure Bacterial Genomic DNA Isolation Kit is designed for rapid isolation of genomic DNA from bacteria. The kit contains a membrane embedded column for binding up to 10µg of genomic DNA. Nucleotides, proteins, salts, and other impurities are washed away. Purified genomic DNA can be used in most molecular biology experiments including restriction enzyme digestion, PCR, Southern-blotting, etc.

Component

Product Component	Quantity
Column & 2.0ml Collection Tube, Use for DNA	50
Universal Buffer Digestion	10ml
Universal Buffer BD	12ml
Universal PW Solution (concentrate)	18ml
Universal Wash Solution (concentrate)	7.5ml
CE Buffer	15ml
Proteinase K	1.2ml

Store columns and solutions at room temperature (15-25°C). Store Proteinase K for up to 6 months at 4°C or long-term at -20°C.

Additional Reagents Supplied by User

1. Isopropanol
2. Ethanol (96-100%)
3. Lysozyme Solution for Gram-positive bacteria (20mg/ml lysozyme, 20mM Tris-HCl, pH 8.0, 2.5mM EDTA, 1% Triton X-100)
4. RNase A (20mg/ml, optional for RNA-free DNA)

Before Use

1. Universal Buffer BD contains a chaotropic salt, avoid contact with skin and eyes.
2. Universal PW Solution and Universal Wash Solution are supplied as concentrates. Add 12ml isopropanol to 18ml Universal PW solution and 22.5ml ethanol (96-100%) for 7.5ml Universal Wash Solution before first use to obtain working solutions, and mark bottles as "diluted".
3. Check the Universal Buffer Digestion and Universal Buffer BD for salt precipitation before each use. If necessary, redissolve the precipitate by warming the solution at 56°C, then cool back down to room temperature before use.
4. CE Buffer is 10mM Tris-HCl, 0.5mM EDTA, pH 9.0. Water can be used to elute the DNA in the final step if EDTA should be avoided for downstream applications, but it is not recommended if the pH of the water is less than 7.0.

Protocol

1. Sample Preparation:
   a. **Gram-negative bacteria** (E. coli, streptococcal, pneumococcal, etc.):
      i. Transfer overnight culture (about 2 x 109 cells) into a 1.5ml centrifuge tube and centrifuge at 10000xg (13000rpm) for 30 seconds, discard supernatant.
      ii. Add 180µl Universal Buffer Digestion and 20µl Proteinase K to the sample, and mix thoroughly by vortexing. Incubate at 56°C for 1 hour.
   b. **Gram-positive bacteria** (golden staphylococcal, ornyebacteriadiphtheriae, etc.):
      i. Transfer overnight culture (about 2 x 109 cells) into a 1.5ml centrifuge tube and centrifuge at 10000xg (13000rpm) for 30 seconds, discard supernatant.
      ii. Add 180µl Lysozyme Solution, suspend thoroughly and incubate at 37°C for 30-60 minutes. Add 20µl Proteinase K and mix thoroughly by vortexing. Incubate at 56°C for 30 minutes.
2. (Optional) If RNA-free genomic DNA is required, add 20µl RNase A (20mg/ml), mix by vortexing, and incubate for 2 minutes at room temperature, then continue with step 3.
3. Add 200µl Universal Buffer BD, mix thoroughly by vortexing. Incubate at 70°C for 10 minutes.
4. Add 200µl ethanol (96-100%), mix thoroughly by vortexing.
   Note: If a gelatinous material appears at this step, vigorously shaking or vortexing is recommended.
5. Transfer the mixture from step 4 (including any precipitate) into the Column-Pure Spin Column placed in a 2.0ml collection tube. Centrifuge at 9000xg (12000rpm) for 1 minute. Discard the flow-through.
6. Add 500µl Universal PW Solution (diluted), and centrifuge at 9000xg (12000rpm) for 1 minute. Discard the flow-through.
7. Add 500µl Universal Wash Solution (diluted), and centrifuge at 9000xg (12000rpm) for 1 minute. Discard the flow-through.
8. Centrifuge the empty column for an additional 2

minutes at 9000xg (12000rpm) to remove residual ethanol from the column membrane. Discard flow-through and transfer the spin column to a clean 1.5ml centrifuge tube.

9. Add 50-100µl Buffer CE directly onto the center of the column membrane. Incubate at room temperature for 1 minute, and centrifuge at 9000xg (12000rpm) for 1 minute to elute the DNA.

General Notes

1. Warming the Buffer CE to 60°C increases the elution efficiency.
2. For maximum DNA yield, repeat elution in step 9. A new microcentrifuge tube can be used for the second elution to prevent dilution of the first eluate.
3. For maximum DNA concentration, reapply the eluate from the first elution to the column membrane for the second elution.

For research use only