Applied Biological Materials Inc.
Tel: 1-866-757-2414
Email: info@abmGood.com
Website: www.abmGood.com

D511: Column-Pure Bacteria Genomic DNA Isolation Kit

Store at 4°C
Store Proteinase K at -20°C

Part No.	Product Components	Quantity
D511-1	Digestion Solution[a]	20 ml
D511-2	Wash Solution[b]	12 ml
D511-3	Elution Buffer[c]	5 ml
D511-4	Proteinase K[d]	2 mg
D511-5	Column-Pure Spin Column (with 2.0-ml Collection Tube)	50
Size		**50 Preps**

a. Digestion Solution may form a precipitate upon storage. Dissolve the precipitate by warming the solution to 37°C if necessary.

b. Before use, add 48 ml of 100% ethanol to 12 ml Wash Solution.

c. The Elution Buffer composition is 2.0 mM Tris-HCl, pH 8.0-8.5. Water can also be used but yield may be slightly lower.

d. Before use, add 150 µl of sterilized water to the tube containing 2 mg of Proteinase K. For long term storage, proteinase K solution should be kept at -20°C.

Introduction

abm's Column-Pure Bacteria 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 10 µg 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.

Protocol

1. Spin bacteria (about 10^6~10^7) at 6,000 x *g* (8,000 *rpm*) for 5 minutes at room temperature. Remove supernatant completely and resuspend cells in 200 µl cold TE (not provided with kit).
2. Add 400 µl of Digestion Solution to 200 µl sample from step 1. Mix well. Add 3 µl of Proteinase K solution (2 mg/150 µl) to sample and incubate at 55°C for 5 minutes.
    - Do not add Proteinase K solution directly to Digestion Solution.
    - Incubation period depends on the nature of sample. For cell cultures, 5 minutes is generally enough to obtain complete lysis.
    - If RNA-free genomic DNA is required, add 20 µl RNase A (10 mg/ml, not provided with kit), mix by vortexing, and incubate for 5 minutes at room temperature before continuing with step 3.
3. Add 260 µl of 100% ethanol, and mix well. Apply the mixture onto a Column-Pure Spin Column that is placed into a Collection Tube. Spin at 8,000 x *g* (10,000 *rpm*) for 2 minutes.
4. Discard the flow-through in the collection tube. Add 500 µl of Wash Solution, and spin at 8,000 x *g* (10,000 *rpm*) for 2 minutes.
5. Repeat step 4.
6. Discard the flow-through. Spin at 8,000 x *g* (10,000 *rpm*) for an additional minute to remove residual amounts of Wash Solution.
7. Place the spin column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl Elution Buffer into the center part of the membrane in the column. Incubate at room temperature for 2 or 3 minutes. Incubating the tube at 37°C or 50°C for 2 minutes may increase recovery yield.
8. Spin at 8,000 x *g* (10,000 *rpm*) for 2 minutes to elute DNA from the column.
9. For long term storage, keep aliquots of purified genomic DNA at -20°C.
10. Measure DNA quantity by UV absorption at A260 (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytical 0.7% agarose gel. The length of genomic DNA is around 50 kb.

Troubleshooting Guide

1. Low yield
    a. Improper storage of starting material
        - Prepare fresh samples and use immediately.
    b. Too much or too little starting material
        - Reduce or increase starting material accordingly.
    c. Incorrect preparation of buffers
        - Each step has to be strictly followed.
2. RNA contamination
    - Perform optional RNase treatment according to the protocol.
3. $OD_{260nm/280nm}$ ratio outside 1.6-2.2 range
    - If the ratio of $OD_{260nm/280nm}$ is greater than 2.2, there may be traces of ethanol present. If the ratio of $OD_{260nm/280nm}$ is smaller than 1.6, there may be protein contamination. Make sure the sample is mixed well after Proteinase K digestion.
4. DNA does not perform well
    a. DNA Shearing
        - Avoid repeated freezing and thawing of starting material; if samples are too old, start with a new sample.
    b. Ethanol Carryover
        - Spin additional steps before elution.

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