TIANamp Bacteria DNA Kit

For isolation of genomic DNA from bacteria

This product is for scientific research use only. Do not use in medicine, clinical treatment, food or cosmetics.

TIANamp Bacteria DNA Kit

(Spin Column)

Cat.no. GDP302

Kit Contents

Contents	GDP302-02 50 preps
Buffer GA	15 ml
Buffer GB	15 ml
Buffer GD	13 ml
Buffer PW	15 ml
Buffer TE	15 ml
Proteinase K	1 ml
Spin Columns CB3	50
Collection Tubes 2 ml	50
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Storage

TIANamp Bacteria DNA Kit should be kept in dry place and can be stored at room temperature (15-30°C) for up to 15 months without showing any reduction in performance and quality. If any precipitate forms in the buffers, it should be dissolved by warming the buffers at 37°C for 10 min before use.

Introduction

TIANamp Bacteria DNA Kit is based on silica membrane technology and provides special buffer system for many kinds sample of gDNA extraction. The spin column is made of new type of silica membrane which can bind DNA optimally on given salt and pH conditions. Simple centrifugation processing completely removes contaminants and enzyme inhibitors such as proteins and divalent cations. Purified DNA is eluted in low-salt buffer or water, and is ready for use in downstream applications.

DNA purified by TIANamp Bacteria DNA Kit is highly suited for restriction enzyme digestion, PCR analysis, Southern blotting, and cDNA library.

Yield For Reference

Bacteria Type	Gram-negative bacteria (such as *E.coll*)	Gram-positive bacteria (such as Gluco-coccus epidermidis)
Bacteria Concentration	2×10^8cells/ml	3.5×10^8cells/ml
Culture Volume	1 ml	1 ml
DNA Yield	15-20 μg	6-13 μg
OD_{260}/OD_{280}	1.7-1.9	1.7-1.9

Note: The DNA extraction amount may vary depending on the bacteria types and culture time,ete.Gram-positive bacteria require special treatments such as lysozyme for lysing,and the genomic DNA extraclion canbe performed according to the procedures of Gram-negative bacteria.

Features

1. **Simple and fast:** Pure genomic DNA of Gram-negative bacteria can be obtained within 1 hour.
2. **Excellent quality:** The purified DNA can be directly used in downstream molecular experiments such as PCR, restriction endonuclease digestion, Southern blotting, etc.

Important Notes <u>Please carefully read before using this kit.</u>

1. Repeated freezing and thawing of stored samples should be avoided, since this leads to DNA size reduction.

2. If precipitates formed in Buffer GA or Buffer GB, warm the buffer to 37°C until the precipitates have fully dissolved.
3. All centrifugation steps should be carried out in a conventional table-top centrifuge at room temperature (15-30°C).

Reagents need to be prepared by Customer

96-100% ethanol, RNase A (100 mg/ml) (optional); Lysozyme (50 mg/ml) (TIANGEN) (optional)

Protocol

Ensure that Buffer GD and Buffer PW have been prepared with appropriate volume of ethanol (96%-100%) as indicated on the bottle and shake thoroughly.

1. Pipet 1-5 ml bacterial culture suspension in a centrifuge tube, centrifuging for 1 min at 10,000 rpm (~11,500 × g), discard supernatant as possible.
2. Add 200 µl Buffer GA. Mix thoroughly by vortex.

   Note: For Gram-positive bacteria, Step 2 could be replaced by lysozyme treatment: add 110 µl enzymatic lysis buffer (20 mM Tris·Cl, pH 8.0; 2 mM sodium EDTA; 1.2% Triton® X-100), and 70 µl lysozyme (50 mg/ml prepared by users). Incubate for at least 30 min at 37°C.

   If RNA-Free genomic DNA is required, add 4 µl RNase A (100 mg/ml, should be prepared by user), mix by vortex for 15 sec, and incubate for 5 min at room temperature (15-30°C).
3. Add 20 µl Proteinase K. Mix thoroughly by vortex.
4. Add 220 µl Buffer GB to the sample, vortex for 15 sec, and incubate at 70°C for 10 min to yield a homogeneous solution. Briefly centrifuge the 1.5 ml centrifuge tube to remove drops from the inside of the lid.

   Note: White precipitates may form when Buffer GB is added. They will not interfere with the procedure and will dissolve during the heat incubation at 70°C. If precipitates do not dissolve during heat incubation, it indicates that the cell is not completely lysed and may result in low yield of DNA and impurity in DNA.
5. Add 220 µl ethanol to the sample, and mix thoroughly by vortex for 15 sec. A white precipitate may form on addition of ethanol. Briefly centrifuge the 1.5 ml centrifuge tube to remove drops from the inside of the lid.

6. Pipet the mixture from step 5 into the Spin Column CB3 (in a 2 ml collection tube) and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard flow-through and place the spin column into the collection tube.
7. Add 500 µl Buffer GD (Ensure ethanol has been added) to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec, then discard the flow-through and place the spin column into the collection tube.
8. Add 600 µl Buffer PW (Ensure ethanol has been added) to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and place the spin column into the collection tube.
9. Repeat Step 8.
10. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min to dry the membrane completely.

    Note: The residual ethanol of buffer PW may affect downstream application.
11. Place the Spin Column CB3 in a new clean 1.5 ml centrifuge tube, and pipet 50-200 µl Buffer TE or distilled water directly to the center of the membrane. Incubate at room temperature for 2-5 min, and then centrifuge for 2 min at 12,000 rpm (~13,400 × g).

    Note: If the volume of eluted buffer is less than 50 µl, it may affect recovery efficiency. The pH value of eluted buffer will have some influence in eluting. We suggest choosing buffer TE or distilled water (pH 7.0-8.5) to elute gDNA. For long-term storage of DNA, eluting in Buffer TE and storing at -30~-15°C is recommended, since DNA stored in water is subject to acid hydrolysis.

    To enhance the gDNA yield, eluate can be reloaded to CB3 once, incubate at room temperature 2min and centrifuge at 12,000 rpm (~13,400 × g) for 2 min to get the final eluate.

Analysis of DNA concentration and purity

Size of gDNA extracted by this kit is related with the sample storage condition, shearing force during operation and some other factors. Purified DNA can be analysed by electrophoresis gel and UV-Spectrophotometer.

DNA has a significant peak at OD_{260}. An OD_{260} of 1 corresponds to 50 µg/ml of dsDNA solution or 40 µg/ml of ssDNA solution.

OD_{260}/OD_{280} ratio value should be within 1.7-1.9. If ddH_2O is used to elute DNA, the ratio value would be lower, since the pH value and the existence of ion could affect the absorption value.