TIANamp Bacteria DNA Kit

For isolation of genomic DNA from bacteria

TIANGEN®

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TIANamp Bacteria DNA Kit

（Spin Column）

Cat. no. DP302

Kit Contents

Contents	DP302-02 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
TIANamp Spin Columns CB3	50
Collection Tubes（2 ml）	50
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Storage

TIANamp Bacteria DNA Kit can be stored dry at room temperature (15-25°C) for up to 12 months without showing any reduction in performance and quality. For longer storage, the kit can be stored at 2-8°C.

Introduction

TIANamp Bacteria DNA Kit is based on silica membrane technology and special buffer system for many kinds of sample's gDNA extraction. The spin column made of new type silica membrane 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, ready for use in downstream applications.

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

Yield of Genomic DNA with TIANamp Genomic DNA Kit

Sample	Volume	DNA yeild
Whole Blood from mammalian	100 µl-400 µl	3-10 µg
Whole Blood from birds or amphibian	5-20 µl	5-40 µg
Cultured cells	10^6-10^7 cells	5-30 µg
Tissue	30 mg	10-30 µg

Important Notes

1. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size.
2. If a precipitate has formed in Buffer GA or Buffer GB, warm buffer to 56°C until the precipitate has fully dissolved.
3. All centrifugation steps should be carried out in a conventional table-top microcentrifuge at room temperature (15–25°C).

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

   Note： For the more difficult broken Gram-positive bacteria, you can skip Step 2, add lysozyme, the specific methods are: add 180 µl enzymatic lysis buffer (20 mM Tris·Cl, pH 8.0; 2 mM sodium EDTA; 1.2% Triton® X-100; Immediately before use, add lysozyme to 20 mg/ml). 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, Cat. No. ： RT405-11), mix by vortexing for 15s, and incubate for 5 min at room temperature (15–25°C).
3. Add 20 µl Proteinase K, mix thoroughly by vortexing.
4. Add 220 µl Buffer GB to the sample, mix thoroughly by vortexing, and incubate at 70°C for 10 min to yield a homogeneous solution. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
5. Add 220µl ethanol (96-100%) to the sample, and mix thoroughly by vortexing for 15s. A white precipitate may form on addition of ethanol. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
6. Pipet the mixture from step 5 into the TIANamp Spin Column CB3 (in a 2 ml collection tube) and centrifuge at 12,000 rpm for 30s. Discard flow-through and place the spin column into the collection tube.
7. Add 500 µl Buffer GD to TIANamp Spin Column CB3, and centrifuge at 12,000 rpm for 30s, then discard the flow-through and place the spin column into the collection tube.
8. Add 700µl Buffer PW to TIANamp Spin Column CB3, and centrifuge at 12,000 rpm for 30s. Discard the flow-through and place the spin

column into the collection tube.

9. Add 500μl Buffer PW to TIANamp Spin Column CB3, and centrifuge at 12,000 rpm for 30s. Discard the flow-through and place the spin column into the collection tube.
10. Centrifuge at 12,000 rpm for 2 min to dry the membrane completely.

    Note: The resident ethanol of buffer PW may have some affection in downstream application.
11. Place the TIANamp Spin Column CB3 in a new clean 1.5 ml microcentrifuge tube, and pipet 50-200 μl Buffer TE or distilled water directly to the center of the membrane. Incubate at room temperature (15–25°C) for 2-5 min, and then centrifuge for 2 min at 12,000 rpm.

    Note: If the volume of eluted buffer is less than 50μl, or it may affect recovery efficiency. What's more, the pH value of eluted buffer will have some influence in eluting, we suggest chose 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 –20°C is recommended, since DNA stored in water is subject to acid hydrolysis.

Ordering Information

RNA Purification

Product	Size	Cat.no.
Lysozyme (50 mg/ml)	1 ml	RT401
Proteinase K (20 mg/ml)	500 μl	RT403-01
	5 ml	RT403-02

PCR MasterMix

Product	Size	Cat.no.
2 × Taq PCR MasterMix	1 ml	KT201-01
(with loading dye)	5 × 1 ml	KT201-02
2 × Taq PCR MasterMix	1 ml	KT201-11
(without loading dye)	5 × 1 ml	KT201-12

DNA Marker

Product	Size	Cat.no.
ΛDNA/Hind III	50 preps	MD202-01
	200 preps	MD202-02