TIANGEN®

# TIANamp Genomic DNA Kit

For isolation of genomic DNA from blood, cells and animal tissues

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# TIANamp Genomic DNA Kit

**(Spin Column)**

**Cat. no. GDP304**

## Kit Contents

| Contents | GDP304-02<br>50 preps | GDP304-03<br>200 preps |
|---|---|---|
| Buffer GA | 15 ml | 50 ml |
| Buffer GB | 15 ml | 50 ml |
| Buffer GD | 13 ml | 52 ml |
| Buffer PW | 15 ml | 50 ml |
| Buffer TE | 15 ml | 60 ml |
| Proteinase K | 1 ml | 4×1 ml |
| Spin Columns CB3 | 50 | 200 |
| Collection Tubes 2 ml | 50 | 200 |
| Handbook | 1 | 1 |

## Storage

TIANamp Genomic DNA Kit can be stored dry 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 Genomic 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 silica membrane which can bind DNA optimally on given salt and pH conditions. Simple centrifugation process 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 DNA library.

## Yield For Reference

| Sample type | Sample amount | DNA yield (μg) |
|---|---|---|
| Mammalian whole blood | 200-500 μl | 3-10 |
| Poultry, amphibian whole blood | 5-20 μl | 5-40 |
| Cultured cells | $10^6$-$10^7$ Cells | 5-30 |
| Animal tissues | 30 mg | 10-30 |
| Mouse tail | 1.2 cm (Tip) | 10-25 |
| Rat tail | 0.6 cm (Tip) | 20-40 |

**Wide adaptability:** Preferred kit for genomic DNA extraction from animal tissues, cells and blood.

**Simple and fast:** the whole experiment can be completed in 1h.

**Highly pure:** the DNA product is of high quality, meeting the needs of various downstream experiments.

## Important Notes

1. Please add ethanol (96-100%) to Buffer GD and Buffer PW as indicated on the bottle for the first use.
2. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size.
3. If a precipitate has formed in Buffer GA or Buffer GB, warm buffer to 37°C until the precipitate has fully dissolved.
4. All centrifugation steps should be carried out in a conventional bench microcentrifuge at room temperature (15-30°C).

## Reagents need to be prepared by Customer

96-100% ethanol, RNase A (100 mg/ml) (TIANGEN, Cat.no GRT405-12) (optional); Red Cell Lysis Buffer (TIANGEN, Cat.no GRT122) (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. Samples preparation:

a. For blood, please use 200 µl fresh, frozen or anticoagulant-adding blood. If less than 200 µl, please make up with Buffer GA to 200 µl.

**Note: If the blood volume is 0.3-1 ml, please refer to the following step: add 3 times volume of Red Cell Lysis Buffer (TIANGEN, Cat.no GRT122) to the sample (e.g., add 900 µl Red Cell Lysis Buffer to 300 µl blood), then close the cap and invert the tube. Stay the tube at room temperature (15-30°C) for 5 min, and centrifuge at 12,000 rpm (~13,400 × g) for 1 min, then discard the supernatant and add 200 µl Buffer GA to the precipitate and mix by pulse-vortex.**

b. If the sample is blood from poultry, birds, amphibians, of which red blood cells have nucleolus, the amount should be reduced to 5-20 µl and made up the volume to 200 µl with Buffer GA.

c. The adherent cells should be treated to cell suspension first, then centrifuge the cells for 1 min at 10,000 rpm (~11,200 × g), then discard the supernatant and re-suspend cell pellet in 20 ul buffer TE or PBS (self provided). Add 180 ul buffer GA to fully resuspend..

d. Animal tissue (spleen<10 mg) should be homogenized first, then centrifuge at 10,000 rpm (~11,200 × g) for 1 min , then discard the supernatant and re-suspend cell pellet in 200 µl Buffer GA.

**Note: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml, TIANGEN, Cat.no GRT405-12), should be prepared by Mix by vortex for 15 s, and incubate for 5 min at room temperature.**

2. Add 20 µl Proteinase K, mix thoroughly.

If the sample is blood and cultured cell, proceed to step 3 after adding Proteinase K.

If the sample is tissue: incubate at 56°C until the tissue is completely

lysed.

**Note: Lysis time varies depending on the type of tissue processed. Lysis usually takes 1-3 h (rat tail needs to be lyzed overnight). Samples should be inverted 2-3 times every one hour, or use Shaking Water Bath.**

3. Add 200 µl Buffer GB to the sample, mix thoroughly, 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.

   **Note: White precipitate 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 of DNA.**

4. Add 200 µl ethanol (96-100%) to the sample, and mix thoroughly for 15 sec. 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.
5. Pipet the mixture from step 4 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.
6. Add 500 µl Buffer GD **(Ensure ethanol (96-100%) 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.
7. Add 600 µl Buffer PW **(Ensure ethanol (96-100%) 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.
8. Repeat Step 7.
9. Centrifuge CB3 with collection tube at 12,000 rpm (~13,400 × g) for 2 min to dry the membrane completely. Put CB3 column at room temperature 2-5 min to air dry the membrane completely.

   **Note: The residual ethanol of Buffer PW may have some affection in downstream application.**

10. Place the Spin Column CB3 in a new clean 1.5 ml microcentrifuge tube, and pipet 50-200 µl Buffer TE 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 elution buffer is less than 50 µl, it may affect recovery efficiency. The pH value of elution buffer will have a great effect on eluting, we suggest using 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.**