

TIANamp Genomic DNA Kit

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

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medicine, clinical treatment, food or cosmetics.

TIANamp Genomic DNA Kit

(Spin Column)

Cat. no. 4992199/4992254

Kit Contents

Contents	4992199 50 preps	4992254 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
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Compatible Reagents

Red Cell Lysis Buffer
RNase A (100 mg/ml)

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 of sample's 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

Source	DNA Yield
Whole blood from mammalian (100-400 μ l)	3-10 μ g
Whole blood from bird or amphibian (5-20 μ l)	5-40 μ g
Cultured cells (10^6 - 10^7 cells)	5-30 μ g
Tissue (30 mg)	10-30 μ g

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).

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 μ l fresh, frozen or anticoagulant-adding blood. If less than 200 μ l, please make up with Buffer GA to 200 μ l.

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 to the sample (e.g., add 900 μ l Red Cell Lysis Buffer to 300 μ l 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 (\sim 13,400 \times g) for 1 min, then discard the supernatant and add 200 μ l 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 μ l and made up the volume to 200 μ l 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 (\sim 11,200 \times g), then discard the supernatant and re-suspend cell pellet in 200 μ l Buffer GA.
- d. Animal tissue (spleen<10 mg) should be treated to cells suspension first, then centrifuge at 10,000 rpm (\sim 11,200 \times g) for 1 min, then discard the supernatant and re-suspend cell pellet in 200 μ l Buffer GA.

Note: If RNA-free genomic DNA is required, add 4 μ l RNase A (100 mg/ml, should be prepared by Mix by vortex for 15 s, and incubate for 5 min at room temperature.

2. Add 20 μ l Proteinase K, mix thoroughly.

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 lysed overnight). Samples should be inverted 2-3 times every one hour, or use Shaking Water Bath.

3. Add 200 μ l 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 μ l 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 ($\sim 13,400 \times g$) for 30 sec. Discard flow-through and place the spin column into the Collection Tube.
6. Add 500 μ l Buffer GD (**Ensure ethanol (96-100%) has been added**) to Spin Column CB3, and centrifuge at 12,000 rpm ($\sim 13,400 \times g$) for 30 sec, then discard the flow-through and place the spin column into the Collection Tube.
7. Add 600 μ l Buffer PW (**Ensure ethanol (96-100%) has been added**) to Spin Column CB3, and centrifuge at 12,000 rpm ($\sim 13,400 \times g$) for 30 sec. Discard the flow-through and place the spin column into the Collection Tube.
8. Repeat Step 7.
9. Centrifuge at 12,000 rpm ($\sim 13,400 \times g$) for 2 min to 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 μ l 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 ($\sim 13,400 \times g$).

Note: If the volume of elution buffer is less than 50 μ l, 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 -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.