TIANGEN®

TIANamp Blood DNA Kit

For isolation of genomic DNA from 0.1-1 ml whole blood

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TIANamp Blood DNA Kit
(Spin Column)

Cat. no. 4992207/4992208

Kit Contents

Contents	4992207 50 preps	4992208 200 preps
Buffer CL	60 ml	250 ml
Buffer GS	15 ml	50 ml
Buffer GB	15 ml	50 ml
Buffer BD	20 ml	80 ml
Buffer GDB	30 ml	120ml
Buffer PWB	15 ml	50 ml
Buffer TB	15 ml	60 ml
Proteinase K	1 ml	4 × 1 ml
Spin Columns CG2	50	200
Collection Tubes 2 ml	50	200
Centrifuge Tubes 1.5 ml	50	200
Handbook	1	1

Compatible Reagents

RNase A (100 mg/ml)

Storage

TIANamp Blood 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. If a precipitate has formed in buffer under 2-8°C, please place the buffer at room temperature or warm at 37°C for 10 min to dissolve the precipitate.

Introduction

TIANamp Blood DNA Kit uses spin column which can specifically bind DNA, and provides special buffer system for effective Blood gDNA extraction. The spin column is made of new type silica membrane which can bind DNA efficiently and specifically. It can maximally remove contaminant proteins and other organic compounds in cells. Genomic DNA isolated with this kit is highly pure, stable and integrated.

Genomic DNA isolated with this product can be served as template for downstream experiments like restriction enzyme digestion, PCR analysis, library construction, Southern blot procedures, chip hybridization and high-throughput sequencing.

Product Features

1. Wide application: The kit can be used to extract gDNA from anticoagulant blood (EDTA, heparin etc.), buffy coat and blood clots directly.
2. High quality: With the unique lysis buffer system, the purified DNA with high concentration, purity and good integrality can satisfy the demand of chip hybridization and high-throughput sequencing.
3. Rapid and non-toxic: The kit uses silica membrane adsorption technology and does not need phenol and chloroform. The whole extraction process can be completed within an hour.

Yield

Sample	Volume (μl)	DNA yield (μg)
Whole Blood from Mammal	100-500	3-10
	500-1000	10-30
Whole Blood from birds or amphibian	5-20	5-40
Blood clots	200-500	1-8
	500-1000	8-15

Important Notes

1. Samples should not be frozen and thawed repeatedly.
2. If precipitate has formed in Buffer GB, dissolve them by incubating at 37°C and mix.
3. All centrifugation steps should be carried out in a conventional bench microcentrifuge at room temperature (15-25°C).
4. If the sample is blood clots, we recommend to use TIANamp Blood Clot DNA Kit or TIANamp Blood DNA kit with liquefation column together.
5. If RNA removal is required, RNase A (100 mg/ml) should be prepared by user.

Protocol

Ensure that ethanol (96-100%) has been added to Buffer PWB as indicated on the bottle.

1. Preparation of blood samples (This kit is designed for 0.1-1 ml blood sample):

   a. If the blood volume is 200 µl, proceed next step directly.

   b. If the blood volume is less than 200 µl, adjust volume to 200 µl with Buffer GS.

   Note: Protocol a and b could be applied for extraction of most 100-200 µl blood sample, but some blood samples with high content of protein, saccharides and lipid or poor storage condition may result in the low ratio of OD260/OD230. Add 1-2.5 times sample volume of Buffer CL could raise OD260/OD230.

   c. If the sample volume is over 200 µl, please refer to following step: add 1-2.5 times volume of Buffer CL to the sample, mix by inverting the tube. Centrifuge at 10,000 rpm (~11,500 × g) for 1 min, then discard the supernatant **(If the lysis is not complete, add 1-2.5 times volume of Buffer CL and repeat the lysis steps once)**. Add 200 µl Buffer GS and mix completely by vortex.

   d. If the sample is blood from poultry, birds or amphibians, of which red blood cells have nucleolus, the sample amount should be reduced to 5-20 µl and adjust the volume to 200 µl by adding Buffer GS.

   e. If the sample is blood clots, use Liquefaction Columns CX1 (not

provided) to liquefy samples, steps are as follows:

e1. Transfer blood clots into Liquefaction Columns CX1, centrifuge at 12,000 rpm (~11,500 × g) for 1 min, collect filtrate **(for large volume of clots, divide sample and centrifuge several times, collect filtrate)**.

e2. Transfer 100 µl-1ml of filtrate and add 1-2.5 times volume of Buffer CL, mix by inverting, centrifuge at 10,000 rpm (~11,500 × g) for 1 min, then discard the supernatant (If the lysis is not complete, add 1-2.5 times volume of Buffer CL and repeat the lysis steps once). Add 200 µl Buffer GS and mix completely by vortex.

Note: If RNA-Free genomic DNA is required, add 4 µl RNase A (100 mg/ml, not provided). Mix by vortex for 15 sec, and incubate for 5 min at room temperature (15-25°C).

2. Add 200 µl Buffer GB and 20 µl Proteinase K to the sample, mix thoroughly by vortex, and incubate at 56°C for 10 min to form a homogeneous solution **(If the mixture does not become clean, extend the incubation time until a homogeneous solution is obtained)**.

   Note: White precipitate may form when Buffer GB is added. It will disappear when incubate at 37°C and not influence downstream experiments. If the solution does not turn clear, it indicates that cells are not completely lysed and may result in low yield and purity. If the sample volume is over 200 µl and do not include Buffer CL lysis step, or the storage condition is poor, the color might become dark brown after water bath, please ensure no block precipitate exists in solution.

   Note: If blood sample has been treated by Buffer CL, add Buffer GB and mix my inverting, then incubate for 5 min at room temperature, then normally high quality genomic DNA can be isolated.

3. Add 350 µl Buffer BD to the sample, mix by inverting and flocky precipitate may form.

4. Pipet the mixture including flocky precipitate from step 3 into Spin Column CG2 (in 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.

5. Add 500 µl Buffer GDB to Spin Column CG2, 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.

6. Add 600 µl Buffer PWB **(Ensure ethanol (96-100%) has been added)**

to Spin Column CG2, 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.

7. Repeat Step 6.

   Note: If blood sample has been treated by Buffer CL, skip step 7.

8. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min to dry the membrane completely. Discard the flow-through, and allow the column to dry for several minutes to dry the membrane.

   Note: Step 8 is aimed to remove residual buffer on spin column. The residual ethanol of buffer will influence the downstream enzyme reaction (enzyme digestion, PCR etc.).

9. Place the Spin Column CG2 in a new clean 1.5 ml microcentrifuge tube, and pipet 50-200 µl Buffer TB to the center of the membrane. Incubate at room temperature (15-25°C) for 2min, and then centrifuge for 2 min at 12,000 rpm (~13,400 × g).

   Note: The volume of elution buffer should not be less than 50 µl, or it may affect the recovery efficiency. Add the solution after centrifuging to Spin Column CG2, incubate at room temperature (15-25°C) for 2 min, centrifuge for 2 min at 12,000 rpm (~13,400 × g). The pH value of elution buffer has a great impact on eluting, we suggest using ddH_2O (pH 7.0-8.5) to elute gDNA. The isolated genomic DNA should be stored at -20°C to avoid degradation.