TIANamp Blood Clot DNA Kit

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

www.tiangen.com/en

TIANamp Blood Clot DNA Kit (0.1-1 ml)

(Spin Column)

Cat. no. 4992855

Kit Contents

Contents	4992855 50 preps
Buffer CL	60 ml
Buffer GS	15 ml
Buffer GB	15 ml
Buffer GD	13 ml
Buffer PW	15 ml
Buffer TB	15 ml
Proteinase K	1 ml
Spin Columns CB3	50
Liquefaction Columns CX1	50
Collection Tubes 2 ml	100
Centrifuge Tubes 1.5 ml	50
Handbook	1

Required Reagents

RNase A (100 mg/ml) (TIANGEN)

Storage

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

Introduction

TIANamp Blood Clot DNA Kit uses silica membrane technology and unique buffer system for blood clot gDNA extraction. The spin column is made of new type silica membrane can be easily bounded by DNA specifically while removing protein and other organic compounds in the cell to as much as possible. The gDNA which extracted by this kit is integrate, pure and stable.

The extracted gDNA with high quality can be used for restriction enzyme digestion, PCR analysis, library construction, Southern blot, etc.

Product Features

Simple and fast: gDNA can be easily extracted within 1 h.

High purity: extracted gDNA can be directly used in biological experiments like PCR, enzyme digestion, molecular hybridization, etc.

Important Notes Please read the notes before use.

1. Ethanol (96-100%) should be added to Buffer GD and Buffer PW as indicated on the bot before use.
2. Samples should not be frozen and thawed repeatedly, or else the integrity and yield of DNA would be affected negatively.
3. If precipitates have been formed in Buffer GB, dissolve them by incubating at 37°C.
4. All centrifugation steps should be carried out in a conventional table-top microcentrifuge at room temperature (15-30°C).
5. If the blood clot sample is more than 1 ml, please purchase TIANGEN TIANamp Blood DNA Midi Kit (4992854) and Liquefaction Columns CX2/CX3.

Protocol

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

   a. Load blood clot samples to Liquefaction Columns CX1, centrifuge at 12,000 rpm (~11,500 × g) for 1 min, collect the flow-through for next step (Blood clot can be prepared separately if the sample volume exceeds the capacity).

   b. Mix the flow-through with 1-2.5 times volume of Buffer CL by inverting. Centrifuge at 10,000 rpm (~11,500 × g) for 1 min, discard the supernatant and keep the cell debris (Step b can be repeated once to ensure a complete lysis procedure). Add 200 µl Buffer GS to the cell debris, mix thoroughly by vortex.

   Note: 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).

2. Add 20 µl Proteinase K, mix thoroughly by vortex.
3. Add 300 µl Buffer GB to the sample, mix thoroughly by inverting, and incubate at 56°C for 10 min to yield a clear solution, during which samples need to be mixed by inverting several times (If the mixture does not clear, please extend the cracking time until the solution is clear).
4. Add 300 µl ethanol (96-100%), and mix thoroughly by inverting. A white precipitate should be formed with the addition of ethanol.
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 replace the spin column into the collection tube.
6. 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 replace the spin column into the collection tube.
7. 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.
8. Repeat Step 7.

9. Set the Spin Column CB3 back to the Collection Tube and 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 air dry with the cap open for several minutes to dry the membrane.

   Note: The purpose of this step is to completely remove residual ethanol which may interfere with down-stream reactions (enzyme digestion and PCR).

10. Place the Spin Column CB3 in a new clean 1.5 ml microcentrifuge tube, and pipet 50-200 µl Buffer TB or distilled water directly to the center of the membrane. Incubate at room temperature (15-30°C) for 2-5 min, 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. In order to get a high yield, flow-through could be pipetted back to the membrane and incubated at room temperature for 2 min, then centrifuge again at 12,000 rpm (~13,400× g) for 2 min. The pH value of elution buffer has a great impact on eluting, we suggest that the pH value should be within the range of 7.0-8.5 if distilled water is used as elution buffer. Low pH value (pH<7) would significantly reduce the efficiency of elution. DNA product should be stored at -20°C to avoid the degradation.