TIANamp N96 Blood DNA Kit

High throughput purification of blood genomic DNA

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TIANamp N96 Blood DNA Kit

(N96 Plate)

Cat. no. 4992450

Kit Contents

Contents	4992450 (4 plates)
Buffer CL	4 × 250 ml
Buffer GB	2 × 50 ml
Buffer GD	2 × 52 ml
Buffer PW	3 × 50 ml
Buffer TB	60 ml
Proteinase K	8 × 1 ml
N96 Plate CB3(H)	4
N96 Well Plate	12
Plate Cover	36
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Storage Conditions:

TIANamp N96 Blood 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

The kit can be used for the purification of fresh and frozen whole blood. The silica gel membrane on the N96 Plate CB3 can specifically adsorb and bind DNA, while removing impurity proteins and other organic compounds in cells to the greatest extent. The extracted genomic DNA fragment is large (up to 50 kb, generally 20-30 kb), high in purity and stable and reliable in quality. 96 samples can be processed simultaneously. The process does not include phenol/chloroform extraction and ethanol precipitation. The kit is also suitable for extracting genomic DNA from cultured cells or animal tissues.

The DNA recovered by the kit is suitable for various conventional operations, including enzyme digestion, PCR, library construction, Southern blot, etc.

Extraction yield

Materials	Sample amount quantity	DNA yield
Whole Blood	200-600 µl	4-20 µg

Important Notes Please Read Before Starting

1. Before using, please add the corresponding volume of ethonal (96-100%) in Buffer GD and Buffer PW according to the label on the bottle.
2. Avoid repeated freezing and thawing of the sample, otherwise the extracted DNA fragments will be small and the DNA yield will be decreased.
3. If there is precipitation in Buffer GB, redissolve in 37°C water bath.
4. All centrifugation steps should be performed at room temperature.
5. **The volume of elution buffer should not be less than 50 µl. Too small volume will affect recovery efficiency.**
6. The pH value of the elution buffer has a great influence on the elution efficiency. If ddH_2O is used as eluent, the pH value should be within the range of 7.0-8.5. If the pH value is lower than 7.0, the elution efficiency will be reduced. The eluted DNA products should be kept at -20°C to prevent DNA degradation.
7. Do not wet the surface of the N96 Well Plate when using the multi-channel pipette. During centrifugation, the Plate Cover must be tightly sealed to prevent cross contamination caused by liquid spillage during centrifugation.

Protocol

(I) 200 µl Blood

Before starting, add 96-100% ethonal to Buffer GD and Buffer PW according to the label on the bottle.

1. Add 20 µl Proteinase K into N96 Well Plate.
2. Add 200 µl blood sample to each well, and mark each sample accordingly.
3. Add 200 µl Buffer GB, pipette up and down for 20 times, and then cover it with a new Plate Cover. Incubate the N96 Well Plate at 56°C for 30 min, and take it out and shake gently every 10 min during the incubation.

   Note: Do not shake violently to prevent cross contamination caused by liquid spillage. When large number of samples are handled, premix Buffer GB and Proteinase K according to the proportion of 20 µl to 200 µl, and the dosage of the premix for each sample is 220 µl.
4. Remove the Plate Cover, add 200 µl ethanol (96-100%), and pipette up and down for 20 times to mix evenly.
5. Add the solution and flocculent precipitate obtained in the previous step into a N96 Plate CB3 (the CB3 is placed in a new N96 Well Plate), cover the plate with a new Plate Cover and centrifuge at 3,600 rpm(~2,130 × g) for 10 min. Discard the waste liquid in the N96 Well Plate, and place the CB3 in the N96 Well Plate again.
6. Add 500 µl Buffer GD to the N96 Plate CB3 **(check whether ethanol has been added before use)**, cover with a new Plate Cover, and centrifuge at 3,600 rpm(~2,130 × g) for 10 min. Discard the waste liquid in the N96 Well Plate, and place the CB3 back on the N96 Well Plate.
7. Add 500 µl Buffer PW to the N96 Plate CB3 **(check whether ethanol has been added before use)**, cover with a new Plate Cover, and centrifuge at 3,600 rpm(~2,130 × g) for 10 min. Discard the waste liquid, and place the CB3 on the N96 Well Plate again.
8. Add 500 µl Buffer PW to the N96 Plate CB3, cover with a new Plate Cover, and centrifuge at 3,600 rpm(~2,130 × g) for 15 min. Discard the waste liquid, and place the CB3 on the N96 Well Plate again.
9. Centrifuge at 3,600 rpm(~2,130 × g) for 10 min to remove the residual Buffer PW from the N96 Plate CB3.

   Note: The residue of ethanol will affect the subsequent enzyme reaction (enzyme digestion, PCR, etc.).

10. Transfer the N96 Plate CB3 into a new N96 Well Plate, add 80 µl of Buffer TB into the middle of the adsorption membrane and incubate at room temperature for 5-10 min. Seal the plate with a new Plate Cover, centrifuge at 3,600 rpm (~ 2,130 × g) for 10 min to collect DNA. Seal with a new Plate Cover, and the DNA can be used for downstream experiments or stored at -20°C.

    Note: The pH value of the elution buffer has a great influence on the elution efficiency. When ddH_2O is used as eluent, the pH value should be within the range of 7.0-8.5, and the elution efficiency will be reduced if the pH value is lower than 7.0. The eluted DNA products should be kept at -20°C to prevent DNA degradation.

(II) 600 µl Blood

Before use, add ethanol (96-100%) to Buffer GD and Buffer PW according to the label on the bottle.

1. Sample treatment

    Add 600 µl blood sample to each Well, and lable each sample accordingly. Add 2 times the sample volume of Buffer CL (the maximum capacity of N96 Well Plate is 2.2 ml) to each well, and pipette up and down for 20 times to mix evenly. Cover the plate with a new Plate Cover, and centrifuge at 3,600 rpm(~2,130 × g) for 10 min. Then remove the Plate Cover, and discard the supernatant.

2. Add Buffer CL with 2 times of sample volume, pipette up and down for 10-20 times to mix evenly, and seal the plate with a new Plate Cover. Centrifuge at 3,600 rpm (~ 2,130 × g) for 10 min, and pipette out the supernatant until remaining about 200 µl of residual solution.

3. Add 20 µl Proteinase K and 200 µl Buffer GB to each well, pipette up and down for 20 times and seal the plate with a new Plate Cover. Incubate at 56°C for 30 min, and take out and shake gently every 10 min during the incubation.

    Note: When large number of samples are handled, premix Buffer GB and Proteinase K according to the proportion of 20 µl to 200 µl, and the dosage of the premix for each sample is 220 µl.

4. Continue with the Step 4 of 200 µl blood extracion workflow.

Detection of DNA Concentration and Purity

The size of the purified genomic DNA fragment is related to factors such as sample storage time and shearing force during the operation. The concentration and purity of DNA fragments can be detected by agarose gel electrophoresis and UV spectrophotometer.

DNA should have a significant absorption peak at OD_{260}, with OD_{260} value of 1 equivalent to about 50 μg/ml double stranded DNA and 40 μg/ml single stranded DNA.

The ratio of OD_{260}/OD_{280} should be 1.7-1.9. If ddH_2O is used for elution, the ratio will be lower because the pH value and the presence of ions will affect the absorption value, but it does not mean low purity.