TIANGEN®

RelaxGene Blood DNA System (0.1-20 ml)

Extraction of genomic DNA from 0.1-20 ml fresh and cryopreserved blood of various anticoagulants

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Cat.no. 4992722/4992723

Kit Contents

Contents	4992722 (For 50 ml blood)	4992723 (For 200 ml blood)
Buffer CLA	125 ml	2×250 ml
Buffer FGA	40 ml	160 ml
Buffer TB	30 ml	60 ml
Proteinase K	250 µl	1 ml
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Storage

This kit can be stored at room temperature (15-25°C) for 12 months. For longer storage, please store 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

This kit adopts a unique buffer system to extract genomic DNA from 0.1-20 ml of fresh and frozen blood with various anticoagulants. The buffer system can remove protein, pigment, lipid and other inhibitory impurities pollution to the greatest extent. The extracted genomic DNA fragment is large, high in yield, good in purity, stable and reliable.

Organic solvents such as phenol and chloroform are not needed in this kit, and the recovered DNA can be suitable for various conventional operations, such as enzyme digestion, PCR, library construction, Southern blot , etc.

Extraction yield (DNA yield varies according to the content of leukocytes in blood samples)

Samples	Storage time	Sample volume (ml)	DNA yield (μg)	OD_{260} / OD_{280}
Human whole blood	One week at 4°C	0.3	3-10	1.7 - 1.9
		1	4-30	1.7 - 1.9
		5	100-200	1.7 - 1.9
		10	200-400	1.7 - 1.9

Features

High yield and high quality: Up to 2-400 μg of high purity DNA can be obtained from 0.1-20 ml of various blood.

Safe and reliable: Organic solvents such as phenol and chloroform are not needed.

High cost-effective: Compared with similar products, the cost performance is high, and the purified DNA samples can be stored for a long time.

Important Notes Please read these notes before using this kit.

1. Repeated freezing and thawing of blood samples will lead to smaller DNA fragments and lower extraction amount. Avoid repeated freezing and thawing of the obtained genomic DNA as much as possible to avoid DNA fragmentation.
2. Storage of blood sample:
   a) Short-term storage: Blood samples containing anticoagulant can be stored at 2-8°C for up to 10 days.

      For the experiments that requires full-length genomic DNA, such as Southern blot, etc. Please store blood samples at 2-8°C for no more than 3 days, at which time the degradation degree of genomic DNA is relatively light.

   b) Long-term storage: Blood containing anticoagulant should be stored at -70°C **(EDTA is recommended as anticoagulant for DNA with high molecular weight).**
3. All centrifugation operations can be performed at room temperature.

Protocol

I. Workflow of small volume whole blood (<600 µl blood samples; Take 300 µl blood as an example)

1. Add 750 µl of Buffer CLA to 300 µl of anticoagulants blood and mix upside down for 20 times.

   Note: For the convenience of matching with centrifuges, add Buffer CLA with the same volume as blood and lyse twice.
2. Centrifuge at 12,000 rpm (~ 13,400 × g) for 1 min, Discard the supernatant, turn the centrifuge tube upside down on clean absorbent paper and stay for 2 min. Ensure that the precipitate remains in the tube **(this step should be handled carefully. In order to prevent the precipitate from being poured out, it is recommended to use a sharp-bottom centrifuge tube).**

   Note: In rare cases, the precipitation may be loose, so the supernatant should be poured slowly, and the centrifuge tube should be inverted on the absorbent paper to reduce the backflow of the supernatant on the tube wall.
3. Prepare the mixture of Buffer FGA and Proteinase K according to Table 1.

   Note: It is best to prepare the mixture when using and use it up within 1 h after mixing.

4. Add 200 µl of the mixture of Buffer FGA and Proteinase K, and immediately shake up and down violently or vortex to mix until the solution is free of lumps.

   Note: When processing multiple samples, immediatly shake up and down violently or vortex to mix after adding the mixture of Buffer FGA and Proteinase K to the sample. For trace amount of colloidal precipitate that's difficult to mix evenly, more Buffer FGA and Proteinase K mixture can be added (see Table 1 for the specific volume) and vortex to mix evenly.

5. Incubate at a 65°C water bath for 10 min, during which mix the mixture several times up and down.

6. Add 200 µl isopropanol and mix up and down for 50 times until filamentous or clustered genomic DNA appears.

   Note: Complete mixing with isopropanol is very important to precipitate DNA. Please carefully observe the solution for the precipitate.

7. Centrifuge at 12,000 rpm (~ 13,400 × g) for 5 min and discard the supernatant. Invert the centrifuge tube on clean absorbent paper. Ensure that the precipitation remains in the tube.

   Note: In rare cases, precipitation may be loose, so the supernatant should be discarded slowly. If the number of white blood cells in the sample is sufficient, white DNA precipitates can be seen.

8. Add 300 µ l of 70% ethanol, vortex for 5 sec, centrifuge at 12,000 rpm (~ 13,400 × g) for 2 min, and discard the supernatant.

9. Place the centrifuge tube upside down on a clean absorbent paper for at least 5 min. Ensure the precipitation in the tube.

   Note: In rare cases, the precipitation may be loose, so the supernatant should be poured slowly, and the centrifuge tube should be inverted on the absorbent paper to reduce the backflow of the supernatant in the tube wall.

10. Air-dry the DNA precipitate until all the liquid is volatilized (at least 5 min).

    Note: The residue of ethanol will affect the subsequent enzyme reaction (enzyme digestion, PCR, etc.). However, excessive drying of DNA precipitation should be avoided, for over-dried DNA is difficult to dissolve.

11. Add 200 µl of Buffer TB , vortex for 5 sec at low speed, and dissolve DNA by heating at 65°C for 20 min, during which flick the DNA softly several times to help the dissolution.

    Note: If insoluble substances exist, the incubation time at 65°C can be extended to 1 h.

II. Workflow of medium volume whole blood (1-10 ml blood samples; Take 5 ml blood as an example)

1. Add 5 ml of Buffer CLA to 5 ml of blood containing anticoagulant, mix it up and down for 20 times, centrifuge at 3, 600 rpm (~2,000 × g) for 2 min, and discard the supernatant.
2. Add 7.5 ml of Buffer CLA to the mixture, mix it up and down for 20 times and centrifuged at 3,600 rpm (~ 2,000 × g) for 2 min. Discard the supernatant, turn the centrifuge tube upside down on clean absorbent paper and stay for 2 min. Ensure that the precipitate remains in the tube **(this step should be handled carefully. In order to prevent the precipitate from being poured out, it is recommended to use a sharp-bottom centrifuge tube).**

   Note: In rare cases, the precipitation may be loose, so the supernatant should be poured slowly, and the centrifuge tube should be inverted on the absorbent paper to reduce the backflow of the supernatant on the tube wall.
3. Prepare the mixture of Buffer FGA and Proteinase K according to Table 1.

   Note: It is best to prepare the mixture when using and use it up within 1 h after mixing.
4. Add 3.3 ml of the mixture of Buffer FGA and Proteinase K to the sample, and immediately shake up and down violently or vortex to mix until the solution is free of lumps.

   Note: When processing multiple samples, immediatly shake up and down violently or vortex to mix after adding the mixture of Buffer FGA and Proteinase K to the sample. For trace amount of colloidal precipitate that's difficult to mix evenly, more Buffer FGA and Proteinase K mixture can be added (see Table 1 for the specific volume) and vortex to mix evenly.
5. Incubate at 65°C in a water bath for 10-30 min, during which mix it several times up and down.

6. Add 3.3 ml isopropanol and reverse it up and down for 50 times until filamentous or clustered genomic DNA appears.

   Note: Complete mixing with isopropanol is very important to precipitate DNA. Please carefully observe the solution for the precipitate.

7. Centrifuge at 3,600 rpm(~2,000 × g) for 8 min and discard the supernatant. Invert the centrifuge tube on a clean absorbent paper. Ensure that the precipitation remains in the tube.

   Note: In rare cases, precipitation may be loose, so the supernatant should be discarded slowly. If the number of white blood cells in the sample is sufficient, white DNA precipitates can be seen.

8. Add 5 ml of 70% ethanol, vortex for 5 sec to mix. Centrifuge at 3,600 rpm (~ 2,000 × g) for 3 min, and discard the supernatant.

9. Place the centrifuge tube upside down on a clean absorbent paper for at least 5 min. Ensure precipitation in the tube.

   Note: In rare cases, the precipitation may be loose, so the supernatant should be poured slowly, and the centrifuge tube should be inverted on the absorbent paper to reduce the backflow of the supernatant in the tube wall.

10. Air-dry the DNA precipitate until all the liquid is volatilized (at least 5 min).

    Note: The residue of ethanol will affect the subsequent enzyme reaction (enzyme digestion, PCR, etc.). However, excessive drying of DNA precipitation should be avoided,because over-dried DNA is difficult to dissolve.

11. Add 500 µl of Buffer TB , vortex for 5 sec at low speed, and dissolce DNA by heating at 65°C for 30 min, during which flick the DNA softly several times to help its dissolution.

    Note: When using a small amount of Buffer TB to dissolve DNA, the incubation time may need to be extended.

III. Workflow of medium volume whole blood (10-20 ml blood samples; Take 10 ml of blood as an example)

1. Sample treatment:

   a. Centrifuge to collect nucleated cells for nucleic acid extraction:

Centrifuge the blood sample at 3,600 rpm (~ 2,000 × g) for 15-20 min, discard the plasma. Add the intermediate buffy coat cells into a 15 ml centrifuge tube, add 10 ml of Buffer CLA, vortex to mix well for 10 sec. Centrifuge at 3,600 rpm (~ 2,000 × g) for 2 min, and discard the supernatant. Add 15 ml of Buffer CLA, and the vortex to mix evenly for 10 sec. Centrifuge at 3,600 rpm (~ 2,000 × g) for 2 min, and discard the supernatant.

b. Using Buffer CLA to treat the blood samples and the collection of the nucleated cells is divided into several times for nucleic acid extraction: Add Buffer CLA and blood samples (ratio 2.5:1) into a 15 ml centrifuge tube, collect multi-times and carry out downstream experiments.

(Take 10 ml of whole blood as an example: Add 5 ml of whole blood and 10 ml of Buffer CLA into two 15 ml centrifuge tubes respectively, mix them up and down for 5 times, centrifuge at 3,600 rpm (~2,000 × g) for 3 min, and discard the supernatant; Add 2.5 ml of Buffer CLA to the mixture, vortex the mixture for 10 sec. Centrifuge at 3,600 rpm (~ 2,000 × g) for 3 min, and discard the supernatant. Then proceed with the downstream operations.)

Note: The Buffer CLA treatment steps of should be handled carefully. In order to prevent the sediment from being poured out, it is recommended to use a sharp-bottom centrifuge tube. In rare cases, the precipitate obtained by Buffer CLA may be very loose, so the supernatant should be poured slowly.

2. Prepare the mixture of Buffer FGA and Proteinase K according to Table 1. For 10-20 ml of whole blood samples, 6.7 ml of Buffer FGA and Proteinase K mixture are required for each sample.

Note: It is best to mix the mixture when using and use it up within 1 h after mixing.

3. Add 6.7 ml of mixture of Buffer FGA and Proteinase K, and immediately shake up and down violently or vortex and mix until the solution is free of lumps.

Note: When processing multiple samples, immediatly shake up and down violently or vortex to mix after adding the mixture of Buffer FGA and Proteinase K to the sample. For trace amount of colloidal precipitate that's difficult to mix evenly, 1 ml more Buffer FGA and Proteinase K mixture can be added and vortex to mix evenly.

4. Incubate at a 65°C water bath for 30 min, during which mix the mixture several times up and down.

   Note: With the digestion of protein, the color of the solution changes from red to yellow-green.

5. Add 6.7 ml isopropanol and mix up and down for 50 times until filamentous or clustered genomic DNA appears.

   Note: Complete mixing with isopropanol is very important to precipitate DNA. Please carefully observe the solution for the precipitate.

6. Centrifuge at 3,600 rpm (~2,000 × g) for 10 min and discard the supernatant. Invert the centrifuge tube on a clean absorbent paper. Ensure that the precipitation remains in the tube.

   Note: If the obtained lump is too loose, prolong the centrifugal time or increase the centrifugal force.

7. Add 10 ml 70% ethanol, vortex for 5 sec, centrifuge at 3,600 rpm (~ 2,000 × g) for 3 min, and discard the supernatant.

   Note: If the obtained lump is too loose, prolong the centrifugal time or increase the centrifugal force.

8. Place the centrifuge tube upside down on a clean absorbent paper for at least 5 min to ensure precipitation in the tube.

   Note: In rare cases, the precipitation may be loose, so the supernatant should be poured slowly, and the centrifuge tube should be inverted on the absorbent paper to reduce the backflow of the supernatant in the tube wall.

9. Air-dry the DNA precipitate until all the liquid is volatilized (at least 5 min).

   Note: The residue of ethanol will affect the subsequent enzyme reaction (enzyme digestion, PCR, etc.). However, excessive drying of DNA precipitation should be avoided, because over-dried DNA is difficult to dissolve.

10. Add 1 ml of Buffer TB, vortex at low speed for 5 sec, and heat at 65°C for 1 h to dissolve DNA. Flick several times during the incubation to assist dissolution.

    Note: If a small volume of Buffer TB is used to dissolve DNA, the incubation time needs to be prolonged.

Table 1 Amount of Various Buffers Required for Different Volumes of Blood (μl)

Reagent	Blood volume (μl)						
	100	300	1000	3000	5000	10000	20000
Buffer CLA	250	750	2500	7500	12500	25000	50000
Buffer FGA	67	200	667	2000	3333	6667	13333
Proteinase K	0.5	1.5	5	15	25	50	100
100% isopropanol	67	200	667	2000	3333	6667	13333
70% ethanol	100	300	1000	3000	5000	10000	20000
Buffer TB	100	200	200	300	500	1000	1000
Additional Buffer FGA and Proteinase K mixture	10	30	100	300	500	1000	1000