Blood DNA Kit

Cat. No.: B1522828 | Pack size: 50T | Storage: Room temperature

Overview

This kit is suitable for rapid and simple extraction of genomic DNA from blood. The extracted genomic DNA has large fragments, high purity, and good stability, and can be directly used in experiments such as PCR, restriction enzyme digestion, and hybridization. The extraction process does not require phenol-chloroform extraction. The blood is lysed with a lysis buffer and digested with proteinase K, then in the presence of absolute ethanol the DNA binds to the WHF spin column. After thorough washing to remove residual proteins, salts, and other impurities, the DNA is finally eluted with Buffer EB.

Kit Contents & Storage

Product No.	Component	Appearance	50T	Storage
B1522828A	Buffer FL	Liquid	60 mL	RT
B1522828B	Buffer DP	Liquid	15 mL	RT
B1522828C	Buffer DBL	Liquid	11 mL	RT
B1522828D	Buffer DBT	Liquid	20 mL	RT
B1522828E	Buffer PW1	Liquid	17 mL	RT
B1522828F	Buffer PW3	Liquid	15 mL	RT
B1522828G	Buffer EB	Liquid	15 mL	RT
B1522828H	Proteinase K	Liquid	1.2 mL	RT
B1522828I	WHF Spin Column (with 2 mL collection tube)	-	1 EA × 50	RT

All components of this kit can be stored under dry conditions at room temperature for 15 months.

Procedure

Before use, add 22 mL of absolute ethanol to Buffer PW1 and 60 mL of absolute ethanol to Buffer PW3.

1. Sample Processing

1.1 Mammalian blood

Take 200 µL of fresh or anticoagulant-treated blood and proceed directly to steps 2-9. If the blood sample volume is less than 200 µL, make up to 200 µL with Buffer DP.

【Note】 If the blood volume to be processed exceeds 200 µL, first add 1-2.5 volumes of Buffer FL to the blood sample, mix by inversion, centrifuge at 12,000 rpm (13,680 × g) for 1 min, and discard the supernatant. If lysis is incomplete, repeat the lysis by adding 1-2.5 volumes of Buffer FL (relative to the original blood volume). After lysis, add 200 µL of Buffer DP to the pelleted nuclei and vortex until thoroughly mixed.

1.2 Avian blood

Take 5-20 µL of fresh or anticoagulant-treated blood and make up to 200 µL with Buffer DP.

1.3 Blood clots

(1) Transfer the blood clot to a GLF liquefaction column (user-supplied). Centrifuge at 12,000 rpm (13, 680 × g) for 1 min and collect the filtrate. If the clot volume is large, pass through the column multiple times and combine the filtrates.

(2) Take 100 µL-1 mL of the collected filtrate, add 1-2.5 volumes of Buffer FL, mix by inversion, centrifuge at 12,000 rpm (13,680 × g) for 1 min, and discard the supernatant. If lysis is incomplete, repeat the lysis by adding 1-2.5 volumes of Buffer FL (relative to the sample volume). After lysis, add 200 µL of Buffer DP to the pelleted nuclei and vortex until thoroughly mixed.

【Note】 If removal of RNA from the extracted DNA is desired, after sample processing add 4 µL of RNase A solution (100 mg/mL) (user-supplied), vortex for 15 sec, and let stand at room temperature for 5-10 min.

2. To the processed 200 µL sample, add 20 µL of Proteinase K and 200 µL of Buffer DBL. Mix thoroughly by inversion and incubate at 56°C for 10 min. The solution should become clear. If it does not become completely clear, extend the incubation time until fully clear.

【Note】 White precipitate may form upon addition of Buffer DBL, but it usually disappears during incubation at 56°C and does not affect subsequent steps. If the blood sample has been pre-treated with Buffer FL, in most cases, after adding Buffer DBL and mixing thoroughly by inversion, let stand at room temperature for 5 min (no heating; invert to mix 1-2 times during this period) to obtain high-quality genomic DNA.

3. Add 350 µL of Buffer DBT and mix thoroughly by inversion. Flocculent precipitate may appear in the solution; this is normal and requires no further treatment.

4. Transfer the solution and the flocculent precipitate from step 3 into a WHF spin column (placed in the provided 2 mL collection tube). Centrifuge at 12,000 rpm (13,680 × g) for 30 sec. Discard the flow-through and place the spin column back into the collection tube.

5. Add 700 µL of Buffer PW1 (with ethanol pre-added) to the spin column. Centrifuge at 12,000 rpm (13,680 × g) for 30 sec. Discard the flow-through and return the spin column to the collection tube.

6. Add 700 µL of Buffer PW3 (with ethanol pre-added) to the spin column. Centrifuge at 12,000 rpm (13,680 × g) for 30 sec. Discard the flow-through and return the spin column to the collection tube.

7. Repeat step 6 to further remove residual impurities on the spin column.

8. Centrifuge the spin column together with the collection tube at 12,000 rpm (13,680 × g) for 2 min to completely remove residual wash buffer (this avoids residual ethanol affecting subsequent DNA elution and experiments).

9. DNA elution

Transfer the spin column to a new 1.5 mL centrifuge tube. Open the cap and let stand for 2 min to allow residual ethanol to evaporate completely. Add 50-200 µL of Buffer EB dropwise to the center of the membrane without touching it. Let stand at room temperature for 1 min. Centrifuge at 12,000 rpm (13,680 × g) for 30 sec. Collect the DNA solution in the centrifuge tube. The DNA can be used immediately for downstream experiments or stored at -20°C for long-term preservation.

【Notes】

(1) If downstream applications are sensitive to pH or EDTA, sterile water can be used for elution instead. The pH of the elution buffer significantly affects elution efficiency. If water is used, ensure its pH is between 7.0 and 8.5 (adjust with NaOH). pH values below 7.0 will reduce elution efficiency.

(2) To increase the final DNA concentration, reload the collected DNA eluate from step 9 onto the same membrane, let stand at room temperature for 2 min, centrifuge at 12,000 rpm (13,680 × g) for 30 sec, and collect the eluate again.

(3) For long-term DNA storage, it is recommended to elute with Buffer EB and store the DNA solution at -20°C to effectively prevent DNA degradation.

DNA Concentration and Purity Assessment

The fragment size of the extracted genomic DNA is influenced by factors such as sample storage time and shear forces during handling. The purified DNA can be assessed for concentration and purity by agarose gel electrophoresis and UV spectrophotometry.

1. Concentration determination

Measure the absorbance of the sample at 260 nm (A_{260}) using a UV spectrophotometer to determine DNA concentration. If dilution is required, Buffer EB is recommended. Dilution with water may lead to inaccurate readings. The absorbance at 260 nm should be kept within 0.1-1.0 to ensure accuracy. Conversion: 1 unit of absorbance at 260 nm corresponds to 50 µg/mL of double-stranded DNA or 40 µg/mL of single-stranded DNA.

2. Purity assessment

Evaluate DNA purity by the A_{260}/ A_{280} ratio. For high-quality genomic DNA, the A_{260}/ A_{280} ratio should be between 1.7 and 1.9. If ddH_2O instead of Buffer EB is used for elution, the ratio may be lower due to the influence of pH and ions on light absorption; this does not necessarily indicate low DNA purity.

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