CYDN03-CHANGYU Blood DNA Midi Kit (Solution Type)

Catalog number	Package
CYDN0301	20 preps × 3 ml
CYDN0302	50 preps × 3 ml

Storage and shipping: 15-25 °C. Stable for 18 months at room temperature.

Description

This kit uses a few rapid steps to extract genomic DNA from whole blood. Red Blood Cell Lysis Buffer first lyses and removes red blood cells, which contain no DNA. Nuclear Lysis Buffer then lyses white blood cells and releases genomic DNA. Protein Precipitation Buffer selectively precipitates and removes protein, and purified genomic DNA is precipitated with isopropanol and redissolved in DNA Dissolution Buffer.

Kit components

Component	Storage	20 preps × 3 ml (CYDN0301)	50 preps × 3 ml (CYDN0302)
10× Red Blood Cell Lysis Buffer	15-25 °C	20 ml	45 ml
Nuclear Lysis Buffer	15-25 °C	60 ml	150 ml
Protein Precipitation Buffer	15-25 °C	20 ml	50 ml
DNA Dissolution Buffer	15-25 °C	10 ml	20 ml

The kit is stable for 18 months at room temperature.

Storage notes

At low ambient temperature, some detergent components of the Nuclear Lysis Buffer may precipitate, making the buffer turbid. Warm in a 37 °C water bath for a few minutes until it becomes clear; do not shake vigorously, as excessive foam may form.

Protein Precipitation Buffer may precipitate. Warm in a 37 °C water bath for a few minutes to help redissolve; if it does not dissolve completely, the buffer is still usable - simply take the upper solution.

Avoid prolonged exposure of reagents to air, which causes evaporation, oxidation and pH changes. Keep all solutions tightly capped after use.

Features

The red blood cell lysis buffer formulation was selected from more than a dozen formulations and lyses quickly and completely.

No toxic reagents such as phenol are required.

Fast and simple: a single sample is usually processed within 1 hour.

Stable results and high yield (typical yield from 3 ml whole blood is 50-150 μg), with a typical OD260/OD280 of 1.7-1.9 and a length of 50 kb-150 kb; the DNA is suitable for library construction, PCR, Southern-blot and restriction digestion.

Notes before starting

Note: All centrifugation steps are carried out at room temperature in a conventional benchtop centrifuge reaching 2,500 x g and fitted with a rotor for 15 ml tubes.

Note: The user must supply isopropanol and 70% ethanol.

Note: Typical yield from 3 ml whole blood is 75-150 μg genomic DNA; white blood cell counts vary considerably between samples, especially clinical samples, so individual yields may vary widely.

Note: The kit is supplied in solution form and can easily be scaled up or down for 20 μl-10 ml whole blood per sample.

Note: The kit can be used with blood collected in various anticoagulants, such as EDTA, citrate and heparin. White cell pellets from heparin-anticoagulated blood are difficult to disperse and lysis may be affected; non-heparin anticoagulants are recommended.

Note: For best results, use fresh blood samples or samples stored at 4 °C for less than 3 days. Do not use samples that have undergone more than 3 freeze-thaw cycles, as this severely reduces yield.

❖ Procedure

Read the notes before starting.

1. Pipette 9 ml 1× Red Blood Cell Lysis Buffer into a 15 ml centrifuge tube.

Before use, dilute the 10× Red Blood Cell Lysis Buffer 10-fold with deionized water to 1×.

2. Equilibrate the anticoagulated whole blood to room temperature and mix by inversion; add 3 ml to the tube containing the red blood cell lysis buffer, invert 6-8 times and flick the tube while inverted to ensure thorough mixing.

3. Leave at room temperature for 10 minutes, inverting and flicking several times to help lyse the red blood cells.

4. Centrifuge at 2,500 x g for 2 minutes. Discard the red supernatant and aspirate as much of the remaining supernatant as possible without disturbing the cell pellet, leaving an intact white blood cell pellet and about 50 μl residual supernatant.

Note: A white blood cell pellet should be visible at the bottom of the tube; some red cell debris may be mixed with it. If most of the pellet is red, red blood cell lysis was incomplete; add an appropriate volume of red blood cell lysis buffer, resuspend the pellet and repeat steps 3 and 4.

5. Vortex until the white blood cell pellet is fully resuspended and dispersed.

Note: Dispersing the white cells is very important for the next lysis step. If lysis buffer is added before the pellet is dispersed, the white cells will not lyse completely and visible clumps will form.

6. Add 3 ml Nuclear Lysis Buffer to the resuspended white cells. Lyse the cells by pipetting up and down, or vortex vigorously for 10 seconds.

7. Optional step: add RNase A (10 mg/ml) to the lysate to a final concentration of 30 μg/ml, mix by inverting 25 times and incubate at 37 °C for 15 minutes to remove residual RNA; then cool to room temperature.

8. Add 1 ml Protein Precipitation Buffer and vortex continuously at high speed for 25 seconds; small protein clumps may be visible after mixing.

9. Centrifuge at 2,500 x g for 5 minutes (the force may be increased if needed). A dark brown protein pellet should be visible at the bottom of the tube, and some protein may float on the surface.

10. Carefully transfer the supernatant (about 3 ml) to a new 15 ml centrifuge tube.

Note: When transferring the supernatant, do not disturb the protein pellet at the bottom or the material floating on the surface. If protein is accidentally transferred, centrifuge again for 2 minutes and take the supernatant.

11. Add an equal volume of room-temperature isopropanol (3 ml) and mix by inverting gently 30 times, or until a cotton-like (fibrous) white DNA precipitate appears.

12. Centrifuge at 2,000 x g for 3 minutes. A white DNA pellet should be visible at the bottom of the tube; discard the supernatant.

13. Add 3 ml 70% ethanol, invert several times to rinse the DNA pellet, centrifuge at 2,000 x g for 1 minute and discard the supernatant (the pellet is loose, so take care not to discard the DNA). Invert the tube, tap it on absorbent paper to drain residual ethanol, remove residual ethanol around the pellet and on the tube wall with a pipette tip if necessary, and air-dry the pellet for several minutes.

Note: Do not over-dry the pellet, or the DNA will be very difficult to dissolve. Do not leave too much ethanol either, as residual ethanol may inhibit downstream reactions such as restriction digestion.

14. Add 250 µl DNA Dissolution Buffer (reduce the volume if a higher concentration is needed) to rehydrate and dissolve the DNA pellet. Mix by flicking the tube. Incubate at 65 °C for 30-60 minutes (no more than 1 hour), or leave at room temperature or 4 °C overnight, flicking the tube occasionally to help rehydration.

Store the DNA at 2-8 °C; for long-term storage, keep at -20 °C.

Troubleshooting

Problem	Comments and suggestions
Clots in the sample	Improper sample storage, insufficient mixing or an unsuitable anticoagulant. Discard clotted samples and collect blood again using EDTA, heparin or citrate as anticoagulant.
Incomplete red blood cell lysis	Blood not equilibrated to room temperature before lysis - equilibrate the sample before processing. Lysis time too short - extend lysis to more than 15 minutes. Insufficient mixing during lysis - invert more often or flick the tube to help lysis.
Low DNA yield	Low white blood cell count in the sample - increase the starting blood volume. White blood cells not lysed completely - follow the operating notes for step 6. Vortex vigorously to disperse and resuspend the white cell pellet before adding the lysis buffer, otherwise lysis is difficult; with heparin-anticoagulated blood the pellet is hard to disperse, so incubate at 65 °C after adding the lysis buffer until no cell clumps are visible. If the number of white cells exceeds the lysis capacity, increase the volume of Nuclear Lysis Buffer. Sample stored too long - yield from blood stored at 4 °C for more than 5 days may be much lower, so do not store samples for long periods. DNA pellet lost during washing - when discarding the supernatant during the 70% ethanol wash, take great care not to discard the DNA pellet.
DNA length less than 20 kb	Blood sample too old or stored incorrectly, causing DNA degradation - use fresh blood samples. Improper handling sheared the genomic DNA - mix gently; do not shake the tube vigorously by hand; use wide-bore tips to transfer or mix DNA.
No protein pellet visible	The lysate was not cooled to room temperature before adding Protein Precipitation Buffer - cool to room temperature or place on ice for 5 minutes before adding the buffer. The buffer was not mixed thoroughly with the lysate - vortex continuously at high speed for 25 seconds; vortexing does not shear the DNA.
A260/A280 below 1.6	Protein contamination - follow the comments and suggestions for No protein pellet visible above and make sure protein is removed by precipitation; also see step 10 to prevent protein contamination. Diluting DNA with water for absorbance measurement lowers A260/A280 - dilute the DNA with TE buffer and ensure that the pH is above 8.0.

DNA pellet difficult to redissolve	The pellet was over-dried - watch the pellet closely while drying and do not over-dry it. Residual ethanol on the tube wall may have evaporated while some water remains; once the pellet at the bottom of the tube is dry, add DNA Dissolution Buffer. Incubate at 65 °C to help redissolution (no more than 1 hour), then leave at room temperature or 4 °C overnight, inverting and flicking occasionally to help dissolution.
Downstream restriction digestion fails	The DNA was not dried completely and too much residual ethanol remains - open the tube and incubate at 65 °C for a few minutes to let the ethanol evaporate.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.