CYDN01-CHANGYU Blood Genomic DNA Mini Kit (Spin Column)

Catalog number	Package
CYDN0101	50 preps
CYDN0102	100 preps
CYDN0103	200 preps

Storage and shipping: 15-25 °C. Stable for 12 months at room temperature. Proteinase K powder should be stored at -20 °C.

Description

This kit is designed for rapid extraction of genomic DNA from whole blood. A unique Binding Buffer CB/proteinase K combination rapidly lyses cells and inactivates cellular nucleases. Genomic DNA then binds selectively to the silica membrane of the spin column under high-ionic-strength conditions. A series of rapid wash and centrifugation steps with Inhibitor Removal Buffer IR and Wash Buffer WB removes cell metabolites, proteins and other impurities, and purified genomic DNA is eluted from the membrane with low-salt Elution Buffer EB.

Kit components

Component	Storage	50 preps (CYDN0101)	100 preps (CYDN0102)	200 preps (CYDN0103)
Buffer BB	15-25 °C	10 ml	20 ml	40 ml
Binding Buffer CB	15-25 °C	15 ml	30 ml	60 ml
Inhibitor Removal Buffer IR	15-25 °C	25 ml	50 ml	100 ml
Wash Buffer WB	15-25 °C	15 ml	25 ml	50 ml
Elution Buffer EB	15-25 °C	15 ml	15 ml	15 ml × 2
Proteinase K powder (optional) 20 mg/ml	-20 °C	20 mg	20 mg × 2	20 mg × 4
Spin Column AC	15-25 °C	50	100	200
Collection Tube (2 ml)	15-25 °C	50	100	200

Before first use, add the indicated volume of absolute ethanol to Wash Buffer WB, mix thoroughly and tick the box on the label to mark that ethanol has been added. The kit is stable for 12 months at room temperature.

Storage notes

Binding Buffer CB or Inhibitor Removal Buffer IR may precipitate at low temperature. Warm in a 37 °C water bath for a few minutes to redissolve; after the buffer becomes clear, cool to room temperature before use.

Proteinase K is supplied as a lyophilized powder to preserve activity and facilitate shipping. After a brief centrifugation, dissolve the powder in 1 ml sterile water. Repeated freeze-thaw cycles may reduce activity; aliquot immediately after dissolution (20 µl per aliquot) and store at -20 °C.

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

Features

No toxic reagents such as phenol are required, and no ethanol precipitation step is needed.

Fast and simple: a single sample is usually processed within 20 minutes.

Repeated column washes ensure high purity. Typical yield from 200 µl whole blood is 3-12 µg, with a typical OD260/OD280 of 1.7-1.9 and a length of 30 kb-50 kb; the DNA is suitable for PCR, Southern-blot and restriction digestion.

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

Typical yield from 200 µl whole blood is 3-12 µg genomic DNA.

Notes before starting

Note: All centrifugation steps are carried out at room temperature in a conventional benchtop centrifuge reaching 13,000 rpm, such as an Eppendorf 5415C or equivalent.

Note: White blood cell counts vary considerably between samples, especially clinical samples, so individual yields may vary widely.

Note: The user must supply isopropanol.

Note: Preheat the required water bath to 70 °C before starting.

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.

Note: Elution Buffer EB contains no EDTA and does not interfere with downstream digestion or ligation. Water may also be used for elution if its pH is above 7.5; a low pH reduces elution efficiency, and DNA eluted with water should be stored at -20 °C. For long-term storage, DNA may be eluted with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), but EDTA may affect downstream restriction digestion, so the eluate may need to be diluted before use.

❖ Procedure

Read the notes before starting. Before first use, add the indicated volume of absolute ethanol to Wash Buffer WB, mix thoroughly and tick the box on the label.

1. Place 200 µl fresh, frozen or anticoagulated blood in a 1.5 ml centrifuge tube.

2. If the starting volume is less than 200 µl, adjust to 200 µl with Buffer BB. If the starting volume is 200-300 µl, scale the reagent volumes up proportionally in subsequent steps. If the starting volume is 300 µl-1 ml, perform red blood cell lysis first (see the appendix).

For anticoagulated blood from birds, amphibians or lower organisms, the red blood cells are nucleated. Use 5-20 µl of blood, adjust to 200 µl with Buffer BB and continue with the subsequent steps.

3. Add 20 µl proteinase K (20 mg/ml) and mix thoroughly. Add 200 µl Binding Buffer CB, vortex immediately and mix thoroughly, and place at 70 °C for 10 minutes. The solution should become clear (although the colour may remain dark).

Optional step (generally not required): if residual RNA must be removed, add 20 µl RNase A (25 mg/ml) before adding the 200 µl Binding Buffer CB, vortex to mix and leave at room temperature for 5-10 minutes.

4. After cooling, add 100 µl isopropanol and vortex immediately and thoroughly; a flocculent precipitate may appear.

Note: Immediate vortexing or pipetting to mix thoroughly in the above steps is very important; incomplete mixing severely reduces yield. If a sample is viscous and difficult to mix, vortex for 15 seconds.

5. Load the mixture from the previous step, including any precipitate, onto Spin Column AC placed in a collection tube. Centrifuge at 13,000 rpm for 30-60 seconds and discard the flow-through.

6. Add 500 µl Inhibitor Removal Buffer IR, centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

7. Add 600 µl Wash Buffer WB (check that ethanol has been added), centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

8. Add 600 µl Wash Buffer WB, centrifuge at 12,000 rpm for 30 seconds and discard the flow-through.

9. Return Spin Column AC to the empty collection tube and centrifuge at 13,000 rpm for 2 minutes to remove residual wash buffer, since residual ethanol can inhibit downstream reactions.

10. Place Spin Column AC in a clean centrifuge tube. Apply 100 µl Elution Buffer EB (prewarmed in a 65-70 °C water bath for best results) to the centre of the membrane, leave at room temperature for 3-5 minutes and centrifuge at 12,000 rpm for 1 minute. Reload the eluate onto the column, leave at room temperature for 2 minutes and centrifuge at 12,000 rpm for 1 minute.

A larger elution volume gives higher elution efficiency. For a higher DNA concentration the elution volume may be reduced, but it should not be less than 50 µl; too small a volume reduces elution efficiency and DNA yield.

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

❖ Appendix: Red blood cell lysis (example: 300 µl and 1 ml whole blood)

1. Place 900 µl red blood cell lysis buffer in a 1.5 ml centrifuge tube, or 3 ml in a 15 ml centrifuge tube.

2. Equilibrate the anticoagulated whole blood to room temperature and mix by inversion. Add 300 µl blood to the 1.5 ml tube and 1 ml blood to the 15 ml tube, 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 during this period to help lyse the red blood cells.

4. Centrifuge at 12,000 rpm for 20 seconds (1.5 ml tube) or at 2,000-3,000 rpm for 5 minutes (15 ml tube). 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 10 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; resuspend the pellet in more red blood cell lysis buffer and repeat steps 3 and 4.

5. Add 200 µl Buffer BB and vortex to resuspend and fully disperse the white blood cell pellet.

White cell pellets from heparin-anticoagulated blood are difficult to disperse, which affects lysis in later steps; non-heparin anticoagulants are recommended for blood collection.

6. Proceed with the main procedure for extraction of genomic DNA from whole blood.

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. 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. Inactive proteinase K - aliquot and freeze after reconstitution and avoid repeated freeze-thaw. Incomplete lysis or incomplete mixing with isopropanol - pipette or vortex immediately after adding Binding Buffer and proteinase K, and immediately after adding isopropanol before loading the column; if the lysate is very viscous, vortex for 15 seconds. Low elution efficiency - make sure step 8 was performed, otherwise residual ethanol reduces elution efficiency; read note 6 and step 9 carefully and elute only with Elution Buffer EB.
DNA cannot be cut or is only partially digested by restriction enzymes	Step 8 omitted, so ethanol inhibited digestion - perform step 8 and air-dry for a few minutes to let residual ethanol evaporate. Silica membrane components co-eluted and inhibited digestion - centrifuge the eluted genomic DNA again at 13,000 rpm for 1 minute and use the supernatant.
DNA length less than 15 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.
Abnormally high A260	Silica membrane components co-eluted and interfered with absorbance - centrifuge the eluted genomic DNA again at 13,000 rpm for 1 minute and use the supernatant.
Slight colour in the eluted DNA solution	Incomplete washing - (1) wash until the centrifuged liquid is colourless; (2) use the eluate as starting material and repeat the procedure, omitting the proteinase K digestion and 70 °C incubation steps.

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