CYDN11-CHANGYU Bacterial Genomic DNA Mini Kit

Catalog number	Package
CYDN1101	50 preps
CYDN1102	100 preps
CYDN1103	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

A unique Binding Buffer/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 (CYDN1101)	100 preps (CYDN1102)	200 preps (CYDN1103)
Buffer RB	15-25 °C	30 ml	60 ml	120 ml
Binding Buffer CB	15-25 °C	11 ml	20 ml	40 ml
Inhibitor Removal Buffer IR	15-25 °C	25 ml	50 ml	100 ml
Wash Buffer WB	15-25 °C	15 ml	25 ml	2 × 25 ml
Elution Buffer EB	15-25 °C	15 ml	20 ml	40 ml
Proteinase K powder (optional) 20 mg/ml	-20 °C	20 mg	2 × 20 mg	4 × 20 mg
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. 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

The silica membrane in the spin columns is a specially manufactured adsorption membrane; adsorption varies very little from column to column and reproducibility is good.

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 30 minutes.

Repeated column washes ensure high purity. Typical OD260/OD280 is 1.7-1.9 and DNA length reaches 30 kb-50 kb; the DNA is suitable for 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 13,000 rpm, such as an Eppendorf 5415C or equivalent.

Note: Preheat the required water bath to 37 °C or 70 °C before starting.

Note: The user must supply isopropanol.

Note: The user must supply 0.5 M EDTA and lysozyme (for Gram-positive bacteria) and lysostaphin (for some Gram-positive bacteria that are difficult to lyse).

Note: Binding Buffer CB and Inhibitor Removal Buffer IR contain irritants. Wear latex gloves and avoid contact with skin, eyes and clothing. If skin, eyes or clothing are contaminated, rinse with plenty of water or saline.

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. Take 0.5-2 ml of culture (no more than 2×10^9 cells), centrifuge at 10,000 rpm for 30 seconds and aspirate the supernatant as completely as possible to collect the cells.

Note: The starting amount can be adjusted according to bacterial density, cell type and expected yield, but the maximum binding capacity of the spin column is 20 µg genomic DNA; an excess of cells above this capacity will severely reduce yield.

2. Add 200 µl Buffer RB to resuspend the cells, centrifuge at 10,000 rpm for 30 seconds and discard the supernatant. Resuspend the cells fully in 180 µl Buffer RB by vortexing or pipetting.

Note: For Gram-positive bacteria that are difficult to lyse, step 2 may be omitted and lysozyme treatment used instead: add 180 µl of buffer (20 mM Tris, pH 8.0; 2 mM Na2-EDTA; 1.2% Triton X-100; add lysozyme to a final concentration of 20 mg/ml immediately before use; the lysozyme must be dissolved in this buffer from lysozyme powder, otherwise it loses activity) and incubate at 37 °C for at least 30 minutes.

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.

Optional step: if residual RNA must be removed, add 4 µl RNase A (100 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 30 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.

Troubleshooting

Problem	Comments and suggestions
Low DNA yield	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. Some Gram-positive bacteria require a specific enzyme for lysis - see step 3 for details and check the characteristics of the bacterial strain.
No DNA obtained	Absolute ethanol was forgotten in Wash Buffer WB - add the indicated volume of absolute ethanol to WB before the first experiment.
Low DNA yield in the eluate	Residual ethanol in the spin column or ethanol at the bottom of the tube - make sure step 9 was performed, otherwise residual ethanol reduces elution efficiency. Water or another non-optimal liquid was used instead of Elution Buffer - read note 6 and step 10 carefully and elute only with Elution Buffer EB.
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 carefully use the supernatant.
DNA cannot be cut or is only partially digested by restriction enzymes	Silica membrane components co-eluted and inhibited digestion - centrifuge the eluted genomic DNA again at 13,000 rpm for 1 minute and carefully use the supernatant. Residual ethanol in the spin column or at the bottom of the tube inhibited digestion - make sure step 9 was performed, then air-dry for a few minutes to let residual ethanol evaporate.

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