CYDN49-CHANGYU Bacteria Genomic DNA Maxi Kit

Catalog number	Package
CYDN4901	10 preps

Storage and shipping: 15-25 °C. Stable for 12 months at room temperature. Proteinase K solution should be stored at 4 °C; it is stable for at least 6 months at up to 25 °C, for 12 months at 4 °C and for 2 years at -20 °C.

Description

A unique Binding Buffer/proteinase K combination rapidly lyses cells. The silica-membrane spin column specifically binds DNA, so no toxic reagents such as phenol or chloroform and no time-consuming alcohol precipitation are required, and protein and other inhibitory impurities are removed to the greatest extent. The kit is suitable for efficient extraction of genomic DNA from many bacterial species. The extracted DNA can be used directly for restriction digestion, PCR, Southern blot and similar experiments.

Kit components

Component	Storage	10 preps (CYDN4901)
Equilibration Buffer	15-25 °C	10 ml
Buffer RB	15-25 °C	120 ml
Binding Buffer CB	15-25 °C	40 ml
Deproteinization Buffer PE	15-25 °C	64 ml
Wash Buffer WB	15-25 °C	50 ml
Elution Buffer EB	15-25 °C	20 ml
Proteinase K solution	4 °C	1 ml × 4
Spin Column XC	15-25 °C	10
Collection Tube (2 ml)	15-25 °C	10

Before first use, add absolute ethanol to Deproteinization Buffer PE and Wash Buffer WB as indicated on the bottle labels and tick the boxes on the labels. The kit is stable for 12 months at room temperature.

Storage notes

Binding Buffer CB or Deproteinization Buffer PE 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 in a ready-to-use glycerol buffer and is shipped at room temperature. After receipt, it can be stored at up to 25 °C for at least 6 months, at 4 °C for 12 months, or at -20 °C for 2 years.

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 time-consuming 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 50 ml-rotor centrifuge reaching 9,000 rpm.

Note: The user must supply ethanol, isopropanol and, optionally, RNase A.

Note: The user must supply 0.5 M EDTA, Triton X-100 and lysozyme (for Gram-positive bacteria).

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

Equilibration Buffer

During long storage, silica-membrane columns may react with charges or dust in the air, which reduces their nucleic acid binding capacity. Pretreatment with Equilibration Buffer greatly reduces the hydrophobic groups on the silica membrane and improves nucleic acid binding, increasing recovery and yield. Equilibration Buffer is a strongly alkaline solution; if it contacts the skin, rinse with plenty of tap water. Keep the bottle tightly capped after use to avoid exposure to air. Store at room temperature.

Usage (pretreat immediately before use): place a new silica-membrane spin column in a collection tube and apply 1 ml Equilibration Buffer to the column. Centrifuge at 8,000 x g (about 9,000 rpm) for 2 minutes, discard the flow-through and return the column to the collection tube. The column is now pretreated; continue with the procedure.

❖ Procedure

Before first use, add absolute ethanol to Wash Buffer WB and Deproteinization Buffer PE as indicated on the bottle labels, mix thoroughly and tick the boxes on the labels.

1. Take 40-60 ml of culture, centrifuge at 8,000 x g (about 9,000 rpm) for 1 minute 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 1 mg genomic DNA; an excess of cells above this capacity will severely reduce yield.

2. Add 8 ml Buffer RB to resuspend the cells, centrifuge at 8,000 x g (about 9,000 rpm) for 1 minute and discard the supernatant. Resuspend the cells fully in 3.6 ml 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 3.6 ml 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 380 µl proteinase K (20 mg/ml) and mix thoroughly. Add 4 ml 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 100 µl RNase A (100 mg/ml) before adding the 4 ml Binding Buffer CB, vortex to mix and leave at room temperature for 15-20 minutes.

4. Optional: pretreat the spin column with Equilibration Buffer as described above. Pretreatment is optional and is recommended for kits that have been stored for a long time.

5. After cooling, add 2 ml isopropanol and vortex immediately and thoroughly; a flocculent precipitate may appear. Transfer the mixture to the column in several portions, centrifuging at 8,000 x g for 1 minute and discarding the flow-through each time.

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 30 seconds.

Note: If the centrifuge rotor has a large tilt angle, load no more than 10 ml of solution onto the column at a time to prevent leakage, until all of the mixture has passed through the column.

6. Add 10 ml Deproteinization Buffer PE (check that ethanol has been added), centrifuge at 8,000 x g (about 9,000 rpm) for 1 minute and discard the flow-through.

7. Add 10 ml Wash Buffer WB (check that ethanol has been added), centrifuge at 8,000 x g (about 9,000 rpm) for 1 minute and discard the flow-through.

8. Repeat the wash step once.

9. Return the empty column to the collection tube and centrifuge at 8,000 x g for at least 3 minutes to dry residual ethanol from the membrane. This step removes residual ethanol completely; residual ethanol inhibits downstream reactions, reduces elution efficiency and lowers DNA yield.

10. Place the column in a new 50 ml centrifuge tube, air-dry at room temperature for 2-3 minutes, apply 1-2 ml Elution Buffer EB (prewarmed in a 65-75 °C water bath to improve yield) to the centre of the membrane, leave at room temperature for 2 minutes and centrifuge at 8,000 x g for 1 minute; discard the column.

To increase DNA recovery, reload the eluate onto the column, leave at room temperature for 1 minute and centrifuge at 8,000 x g for 1 minute. Two elution passes increase the concentration by about 10%.

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 600 µl; too small a volume reduces elution efficiency and DNA yield.

Store the DNA at -20 °C; for long-term storage, keep at -70 °C.

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