CYDN13-CHANGYU Midi/Maxi Bacterial Genomic DNA Kit

Catalog number	Package
CYDN1301	20 preps
CYDN1302	50 preps

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

Description

This kit is designed for rapid extraction of genomic DNA from various bacteria. Nuclear Lysis Buffer is added to the bacterial sample (after the cell wall has been digested with lysozyme or other enzymes where necessary). Cells are lysed by a strong detergent, releasing genomic DNA. RNase A is then added to remove RNA, 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 (CYDN1301)	50 preps (CYDN1302)
Nuclear Lysis Buffer	15-25 °C	90 ml × 2	250 ml × 2
Protein Precipitation Buffer	15-25 °C	60 ml	150 ml
DNA Dissolution Buffer	15-25 °C	10 ml	20 ml
RNase A (10 mg/ml)	-20 °C	500 µl	1 ml

The kit is stable for 12 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 with gentle swirling 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

No toxic reagents such as phenol are required.

Fast and simple: a single sample is usually processed within 30 minutes.

Stable results and high yield, 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 3,000 x g and fitted with a rotor for 50 ml tubes.

Note: The user must supply isopropanol, 70% ethanol, 0.5 M EDTA, lysozyme (for Gram-positive bacteria), lysostaphin (for some Gram-positive bacteria that are difficult to lyse) and a water bath.

Note: Preheat the required water bath before starting.

Note: The kit is supplied in solution form and can easily be scaled up or down for different amounts of bacterial cells.

❖ Procedure

Read the notes before starting.

1. Collect 10 ml of overnight bacterial culture into a 15 ml centrifuge tube.

2. Centrifuge at 2,000 x g for 3 minutes to pellet the cells, discard the supernatant and disperse the pellet by vortexing or flicking. For Gram-positive bacteria continue with step 3; for Gram-negative bacteria go directly to step 6.

3. Add 4.8 ml 50 mM EDTA (pH 8.0) to resuspend the cells completely.

4. Add 1.2 ml lysozyme (20 mg/ml) and mix.

Note: Most Gram-positive bacteria, such as Bacillus subtilis, Micrococcus luteus, Arthrobacter luteus, Nocardia otitidiscaviarum, Rhodococcus rhodochrous and Brevibacterium albidum, are effectively lysed by lysozyme. For some Staphylococcus species, however, add 60 µl lysozyme (20 mg/ml) and 60 µl lysostaphin (20 mg/ml) to ensure effective lysis.

5. Incubate at 37 °C for 30-60 minutes. Centrifuge at 2,000 x g for 10 minutes, discard the supernatant and disperse the pellet by pipetting or vortexing.

6. Add 9 ml Nuclear Lysis Buffer to the dispersed cells and lyse them by gentle pipetting.

7. Incubate at 80 °C for 5 minutes to lyse the cells, then cool to room temperature.

8. Add 18 µl RNase A (10 mg/ml) to the lysate to a final concentration of 20 µg/ml. Mix by inversion and incubate at 37 °C for 15-60 minutes to remove residual RNA. Then cool to room temperature for at least 5 minutes.

9. Add 3 ml Protein Precipitation Buffer to the lysate at room temperature and vortex continuously at high speed for 25 seconds; small protein clumps may be visible after mixing. Incubate on ice for 5 minutes.

Note: Vortex mixing of these small samples does not shear genomic DNA. If mixing by hand, do not shake the tube vigorously up and down; shake with moderate force, but not so gently that the viscous lysate is not dispersed, otherwise DNA cannot separate from the protein pellet, may be lost with the protein during centrifugation and yield may fall. Incomplete mixing may also leave large amounts of protein in the final product. Vortex mixing is therefore recommended.

10. Centrifuge at 5,000 x g for 10 minutes (the force may be increased if needed). A protein pellet should be visible at the bottom of the tube, and some protein may float on the surface.

11. Carefully transfer the supernatant to a new 50 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 5 minutes and take the supernatant.

12. Add an equal volume of room-temperature isopropanol (about 9 ml) and mix by inverting gently 30 times, or until a cotton-like (fibrous) white DNA precipitate appears.

Note: During inversion, the cotton-like (fibrous) DNA sometimes sticks to the cap or the rim of the tube and does not move down even when the tube is inverted, so no precipitate is visible and it may appear that no DNA was obtained. To solve this, omit step 13, centrifuge directly at 2,000 x g for 5 minutes, discard the supernatant and then continue with step 15.

13. Stand the tube upright and let the white DNA precipitate settle to the bottom; then aspirate and discard as much supernatant as possible without disturbing the precipitate.

14. Add 9 ml 70% ethanol, invert several times to rinse the DNA pellet, centrifuge at 2,000 x g for 3-5 minutes and discard the supernatant; a white DNA pellet should be visible at the bottom of the tube.

15. Add 5 ml 70% ethanol, invert several times to rinse the DNA pellet, centrifuge at 2,000 x g for 1 minute and discard the supernatant (take care not to discard the DNA pellet). 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.

16. Add 500 µl DNA Dissolution Buffer 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), flicking occasionally to help rehydration; alternatively leave at room temperature or 4 °C overnight, inverting and flicking occasionally.

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

Troubleshooting

Problem	Comments and suggestions
Low DNA yield	An inappropriate lysis buffer was used, causing incomplete lysis - do not overload the sample material. Some Gram-positive bacteria are difficult to lyse - use lysostaphin as described in step 4. The lysate was not mixed thoroughly with Protein Precipitation Buffer, so DNA could not separate from the precipitated protein and was lost during centrifugation - see step 9 and mix thoroughly. DNA pellet lost during washing - when discarding the supernatant during the 70% ethanol wash, take great care not to discard the DNA pellet.
A260/A280 above 1.9	Insufficient RNase treatment leaves RNA contamination - increase the amount of RNase or extend the treatment time to 1 hour. DNA was sheared - follow the procedure strictly and avoid vigorous handling.
A260/A280 below 1.6	High residual protein - ensure sufficient lysis buffer volume and time; see the comments and suggestions for No protein pellet visible below and make sure protein is removed by precipitation; also see step 11 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 not fully dissolved - 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.
Discoloured DNA	If the 70% ethanol rinse is not performed promptly after isopropanol precipitation, DNA from some tissues such as liver may become discoloured - perform the 70% ethanol wash immediately after the isopropanol precipitation and centrifugation.
DNA length less than 20 kb	Sample too old, stored incorrectly or repeatedly freeze-thawed, causing DNA degradation - use fresh 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. The mixture was not placed on ice for 5 minutes after adding the buffer - place on ice for 5 minutes before centrifugation to help precipitation.
DNA pellet difficult to redissolve	The pellet was over-dried - watch it 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 or PCR is inhibited	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.