Plasmid Mini Kit

Cat.:BR2D101

V01

Product Description

The Plasmid Mini Kit uses the alkaline lysis method to lyse cells. It employs a unique silica membrane spin column that specifically binds plasmid DNA under high-salt and low-pH conditions, removing proteins, RNA, genomic DNA, and other impurities to yield high-quality, high-purity plasmid DNA. Each spin column can bind up to 30 μg of plasmid DNA, making it suitable for the efficient extraction of plasmid DNA from 1–5 mL of overnight-cultured Escherichia coli. The yield and quality of the extracted plasmid are affected by factors such as host strain type and culture conditions, cell lysis efficiency, plasmid copy number, and plasmid stability. Plasmid DNA extracted using this kit can be directly used in various routine molecular biology experiments, including restriction digestion, ligation, PCR, sequencing, and transformation.

Components

Components	BR2D101-01 50T	BR2D101-02 200T
Buffer P1	15 mL	60 mL
Buffer P2	15 mL	60 mL
Buffer P3	20 mL	80 mL
Buffer PD	30 mL	120 mL
Buffer PW	15 mL	50 mL
Elution Buffer	15 mL	30 mL
Spin Columns with Collection Tubes	50 sets	200 sets
RNase A（10 mg/mL）	150 μL	600 μL

Storage

The kit is stored dry at room temperature (15–30°C) for 18 months. If precipitation occurs in the solutions, preheat in a 37°C water bath for 10 min before use to dissolve the precipitate, which does not affect performance. RNase A is stored at −20±5°C for 18 months. Prior to first use, add all RNase A into Buffer P1, mix well, and store at 2–8°C for stable storage up to 6 months.

Materials

Anhydrous ethanol

Notes

1. For Research Use Only. Not for use in diagnostic procedures.
2. Prior to first use, Buffer PW must be diluted with 4 volumes of absolute ethanol (e.g., add 60 mL absolute ethanol to 15 mL Buffer PW; add 200 mL absolute ethanol to 50 mL Buffer PW) and stored at room temperature. Incorrect dilution or failure to dilute will prevent DNA from binding to the column, resulting in extraction failure.
3. Prior to first use, add RNase A to Buffer P1 (add all the RNase A supplied with the kit), mix well, and store at 2–8°C.
4. Avoid direct skin contact with Buffer P2 and Buffer P3. Cap the bottles tightly immediately after use to prevent changes in pH.
5. The quality of the extracted plasmid is related to factors such as bacterial culture concentration and plasmid copy number.
6. The culture time of the bacterial liquid should not be too long, and the volume should not exceed 5 mL. If the bacterial density is high, the volumes of Buffer P1, P2 and P3 should be increased proportionally as appropriate to avoid overloading.
7. The larger the elution volume, the higher the elution yield. To obtain plasmid at a higher concentration, the elution volume may be appropriately reduced, but the minimum volume should be no less than 30 μL. An excessively small volume will reduce elution efficiency.

Protocol

Note: Before the first use, add 4 volumes of absolute ethanol to Buffer PW. Add RNase A to Buffer P1 before its first use.

1. Take 1–5 mL of overnight bacterial culture, transfer to a centrifuge tube, centrifuge at 12,000 rpm for 1 min, and discard the supernatant (remove as much supernatant as possible; bacteria can be collected into one centrifuge tube by repeated centrifugation if needed).
2. Add 250 μL of Buffer P1 to the centrifuge tube containing the bacterial pellet (check that RNase A has been added before use), and mix thoroughly using a vortex mixer to ensure complete resuspension of the bacterial pellet.
Note: Undissolved bacterial clots will impair lysis and result in low yield and purity.
3. Add 250 μL of Buffer P2 to the centrifuge tube, and gently invert 6–8 times to fully lyse the bacteria.
Note: Do not vortex vigorously, as this may shear genomic DNA and contaminate the plasmid. The bacterial suspension will become clear and viscous at this stage. Lysis time should not exceed 5 min to avoid plasmid damage. If the suspension does not become clear, it may indicate insufficient lysis due to excessive bacterial biomass, in which case the bacterial volume should be reduced.
4. Add 350 μL of Buffer P3, immediately invert gently 6–8 times and mix thoroughly. A white flocculent precipitate will appear at this point. Centrifuge at 12,000 rpm for 10 min.
Note: Mix immediately after adding Buffer P3 to avoid local precipitation. If tiny white precipitates remain in the supernatant, centrifuge again before transferring the supernatant.
5. Transfer the supernatant obtained from the previous centrifugation into the adsorption column using a pipette, taking care not to aspirate any precipitate. Centrifuge at 12,000 rpm for 1 min and discard the waste liquid.
6. Optional step: Add 500 μL of Buffer PD, centrifuge at 12,000 rpm for 1 min, and discard the waste liquid.
Note: This step is recommended if the host strain is endA$^+$ (such as TG1, BL21, HB101, JM series, ET12567, etc.), as these strains contain high levels of nucleases that can easily degrade plasmid DNA. If the host strain is endA$^-$ (such as DH5α, TOP10, etc.), this step can be omitted.
7. Add 600 μL of Buffer PW (check that absolute ethanol has been added before first use), centrifuge at 12,000 rpm for 1 min, and discard the waste liquid. Repeat this step.
8. Centrifuge at 12,000 rpm for 2 min to completely remove residual Buffer PW.
9. Place the adsorption column into a clean centrifuge tube, open the lid and let stand at room temperature for 2 min. Add 50–100 μL of elution buffer or deionized water to the center of the adsorption column (preheating at 60–70°C in advance will improve elution efficiency) and let stand for 1 min. Centrifuge at 12,000 rpm for 2 min to recover the plasmid DNA, and store at −20°C. To increase the plasmid concentration, it is recommended to add the resulting solution back into the adsorption column and repeat this step for a second elution.

FAQs

1. Low yield of extracted plasmid DNA:
Low plasmid copy number results in low extraction yield; a high-copy vector with the same function may be used instead. When extracting low-copy plasmids or large plasmids over 10 kb, increase the bacterial volume by using 5–10 mL of overnight culture, and double the volumes of Buffer P1, P2 and P3. Insufficient alkaline lysis leads to low concentration; reduce the bacterial culture volume or increase the amounts of Buffer P1, P2 and P3.
2. Genomic DNA contamination:
Excessively long bacterial culture time; culture duration should be controlled within 12–16 h. Mix gently when adding Buffer P2 and P3; vigorous vortexing may shear genomic DNA and contaminate the plasmid. When processing multiple samples simultaneously, the total time starting from the addition of Buffer P2 should not exceed 5 min.
3. Low elution efficiency:

Preheat the elution buffer to 60–70°C and perform a second elution.

4. Salt contamination:
Wash twice with Buffer PW. Buffer PW can be applied along the inner wall of the adsorption column. After addition, cap the tube and invert to mix 2–3 times to help thoroughly remove residual salt on the column wall.