High Purity Plasmid DNA Mini-Extraction Kit

Cat. No.

PE116-200

Composition

Contents	Amount(200 rxns)
RNase A（100 mg/mL）	60 μL
Buffer BL	60 mL
Buffer P1	60 mL
Buffer P2	60 mL
Buffer P3	80 mL
Buffer W1	2×72 mL
Elution Buffer	25 mL
Adsorption column EC	200

Storage Condition

Store at room temperature (10~25℃) for 12 months, and store Buffer P1 after adding RNase A should be stored at 2~8℃.

Introduction

This kit adopts the improved SDS-alkali lysis method, combined with the advanced silica gel membrane adsorption technology, to achieve the purpose of rapid purification of plasmid DNA. It is suitable for extracting up to 20 μg of high-purity plasmid DNA from 1~4 mL of bacterial culture. The extracted plasmid DNA can be used for molecular biology experiments such as enzyme digestion, PCR, sequencing, bacterial transformation, in vitro transcription and translation, etc.

Highlights

1. Easy to operate: Plasmid DNA extraction from multiple samples can be completed within 30 min.
2. High efficiency: more than 85% of plasmid DNA can be extracted from bacterial cells.

Usage

This product is suitable for extracting up to 20 μg of high-purity plasmid DNA from 1~4 mL of bacterial culture.

Imporatant Notes

1. Add RNase A to Buffer P1 before use (add all the RNase A provided in the kit), mix well and store at 2~8℃.
2. Before the first use, add absolute ethanol to Buffer W1, see the label on the bottle.
3. When the ambient temperature is low, the SDS in Buffer P2 may appear turbid or precipitate. It can be recovered by heating it in a 37°C water bath for a few minutes. Do not shake vigorously to avoid foam formation.
4. Buffer P3 contains irritating solution, wear latex gloves, masks and glasses during operation. In case of contact with skin and eyes, rinse immediately with plenty of water or normal saline, and seek medical attention if necessary.
5. Please close the lid immediately after each solution is used.
6. The yield and quality of grain extraction are related to the type of host bacteria, plasmid copy number, plasmid stability and other factors.

Protocol

1. Add 250 μL of Buffer BL to the adsorption column EC, and centrifuge at 12,000 × g for 1 min to activate the silica membrane.
2. Take 1~4 mL of overnight cultured bacterial solution, centrifuge at 12,000 × g for 1 min, collect the bacterial cells, and remove the supernatant as much as possible.
3. Add 250 μL Buffer P1 (please check whether RNase A has been added first) to resuspend the cell pellet (if the cell pellet is not completely suspended, it will affect the lysis effect, resulting in low extraction yield and purity), vortex and shake until no until the bacteria block.
4. Add 250 μL Buffer P2, and gently turn up and down 6~8 times to fully lyse the cells.

Note: This step requires gentle inversion, not violent shaking, so as not to interrupt the genomic DNA, so that the extracted plasmid contains genomic DNA fragments. After mixing, the bacteria should become clear and viscous. If it does not become clear, it may be due to the excessive amount of bacteria and insufficient lysis, and the amount of bacteria should be reduced.

5. Add 350 μL Buffer P3, gently turn up and down 6~8 times, mix well (white flocculent precipitate will appear at this time), and centrifuge at 12,000 ×g for 10~15 min.
6. Carefully aspirate the supernatant, transfer the supernatant to the adsorption column EC (be careful not to aspirate the precipitate), centrifuge at 12,000 × g for 1 min, discard the waste liquid, and put the adsorption column EC back into the empty collection tube.
7. Add 700 μL of Buffer W1 to the adsorption column EC (please check whether the specified volume of absolute ethanol has been added), centrifuge at 12,000 × g for 1 min, and discard the waste liquid.

8. Repeat step 7.

9. Put the adsorption column EC back into the empty collection tube and centrifuge at 12,000 × g for 2 min.

10. Take out the adsorption column EC, put it into a clean 1.5 mL centrifuge tube, and let it stand at 20~25°C for 2 min to evaporate the residual ethanol. Add 35~50 μL of Elution Buffer to the middle of the adsorption membrane (preheating Elution Buffer at 60~65°C is better), let stand at 20~25°C for 2 min, and centrifuge at 12,000 × g for 2 min. If more DNA is needed, the obtained solution can be transferred back to the adsorption column EC and centrifuged for 2 min.

Note: The larger the elution volume, the higher the elution yield. If a higher concentration of DNA is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 25 μL. If the volume is too small, the DNA elution yield will be reduced. rate, reducing production. The pH of the eluent has a great influence on the elution efficiency. If subsequent sequencing is performed, it is recommended to elute with ddH_2O, and ensure that its pH value is in the range of 7.0~8.5. If the pH value is lower than 7.0, the elution efficiency will be reduced; and the DNA product should be stored at -20°C to prevent DNA degradation.

Q&A

Q1: Why is plasmid DNA yield low?

A1:

1) Plasmid copy number is low. Vectors can cause significant fluctuations in plasmid yield due to differences in copy number. High-copy-number vectors often fluctuate by 2~3 times the yield (3~16 μg of high-copy-number plasmid vectors per milliliter of bacterial culture cultured overnight). Long fragment plasmids and expression vectors are often dominated by medium and low copy numbers, and the yield per milliliter of bacterial solution is about 0.5~2 μg.

   - Low-copy plasmids: pBR322, pACYC and its derivatives, pSC101 and its derivatives, SuperCos, pWE15.
   - High copy plasmids: pTZ, pUC, pBS, pGM-T.

2) Bacteria problem. Plasmids are lost in the process of strain preservation. It is best to streak and activate before culturing bacteria to stabilize the yield.

3) Bacteria are not sufficiently lysed. Bacteria must be fully resuspended in Buffer P1/RNase A, as agglomerated bacteria cannot be lysed, which will reduce the yield.

4) The reagent preparation is wrong. If there is precipitation in Buffer P2, it needs to be heated to dissolve, and the volume of ethanol added to Buffer W1 is not accurate.

5) The elution efficiency is low. Preheat the Elution Buffer to 60~65℃, and perform the second elution.

Q2: Why is there genomic DNA contamination in plasmid DNA?

A2:

1) The bacterial culture time is too long. Bacterial liquid culture time should be controlled within 12~16 h.

2) The cracking problem. When adding Buffer P2, it must be mixed by gentle inversion; when processing multiple samples, the total time should not exceed 5 min after adding Buffer P2.