ELK Biotechnology

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Endo-Low Plasmid Prepare Kit

Catalog No.	Specification	Storage/Shelflife
EP015-50T	50T	Room temperature/1 year
EP015-200T	200T	Room temperature/1 year

Introduction

This kit adopts an improved SDS alkaline lysis method, combined with a method of preparing a membrane with high adsorption amount of DNA to selectively adsorb DNA to achieve the purpose of rapid purification of plasmid DNA. With the special Endo-Remove Buffer, it can effectively remove endotoxins. It is suitable for extracting up to 60 μg of high-purity plasmid DNA from 5-30 ml of bacterial culture for molecular biology experiments such as sequencing, transfection, in vitro transcription and translation, restriction endonuclease digestion, and bacterial transformation.

Kit Components

Components	EP015-50T	EP015-200T	Storage
Solution A	25 ml	100 ml	RT
Solution B	25 ml	100 ml	RT
Solution C2	35 ml	140 ml	RT
Wash Buffer	60 ml	240 ml	RT
Endo-Remove Buffer	9 ml	36 ml	2-8℃
Elution Buffer	10 ml	40 ml	RT
RNase A	125 μl	500 μl	-20℃
Adsorption column P column	50 sets	200 sets	RT
User Manual	1 copy	1 copy	RT

Before starting

Solution A: After adding RNaseA to the provided Solution A, please store Solution A at 4 ℃.

Solution B, C2: sealed stored. If Solution C2 has not been used for a long time after opening the

lid, please check the pH of Solution C2 to ensure that the pH is ≤ 4.8. If the pH is too high, add a small amount of acetic acid to adjust it.

Wash Buffer: Please add absolute ethanol to Wash Buffer as required before use (there is a label on the reagent bottle).

Endo-Remove Buffer: It needs to be stored at 2-8°C for long-term storage.

Operationsteps

1. Inoculate the strains to 5-30 ml of liquid medium, and culture at 37 ℃ for 12-16 h with shaking. Centrifuge at 13,000 g for 1 min at room temperature, collect the bacteria, and aspirate the supernatant as much as possible.

Note:

a. The remaining liquid medium will easily lead to insufficient lysis of the bacterial solution. After the fifth step of centrifugation, the precipitate is loose and the supernatant cannot be effectively absorbed.

b. The operating procedures in this manual are applicable to the bacterial solution with the OD_{600} (bacterial density) of the culture medium between 2.0-3.0 after culturing in standard LB (Luria Bertani) medium for 12-16 h. If you are using an enriched medium, such as TB or 2×YT, please ensure that the OD_{600} does not exceed 3.0.

2. Add 500 µl Solution A and vortex to fully suspend the bacterial cells.

Note: If the bacterial cells are not fully suspended uniformly, it will result in incomplete lysis of the bacteria, thereby reducing the yield.

3. Add 500 µl Solution B, and gently invert and mix 5-10 times to mix evenly. At this time, the solution is viscous and clear.

Note: Do not shake vigorously. The time for this step should not exceed 5 minutes. Excessive time will cause genomic DNA contamination or plasmid damage. If the solution is not clear and clarified, it indicates that the cell lysis is insufficient, and the amount of Solution B should be increased or the amount of cells should be reduced.

4. Add 700 µl Solution C2 and mix by inversion for 5-10 times. At this time, a white flocculent precipitate appears.

5. Transfer the centrifuge tube to a high-speed centrifuge, and centrifuge at 12,000 rpm (≈13,000×g) at room temperature for 10 min (if white precipitate floating in the supernatant, centrifuge again). Carefully aspirate the supernatant after centrifugation.

6. Add 1/10 times the volume of Endo-Remove Buffer to the supernatant (for example, if the

supernatant is 500μl, add 50μl of Endo-Remove Buffer).

7. Ice bath for 10 minutes, mix upside down and mix several times during the ice bath to complete. (After adding Endo-Remove Buffer, the mixed system may become turbid, it should be clear after ice bath)

8. Incubate at 42℃ for 5-10min, during which it can be turned upside down several times. (At this time, the solution will appear turbid again)

9. Centrifuge at 12,000 rpm (≈13,000×g) for 5 minutes at room temperature. At this time, the solution will be stratified. Carefully pipette the supernatant into a new 1.5 ml EP tube.

Note: the temperature in this step must be above 20°C, otherwise there will be no stratification and the endotoxin cannot be removed. For refrigerated centrifuges, it is recommended to adjust to 25°C in advance.

10. Add 3 times the volume of absolute ethanol, mix upside down for 6-8 times, and let it stand at room temperature for 1-2 minutes.

11. Add the supernatant obtained in the previous step to the P column of the adsorption column provided by this kit (if the addition cannot be completed at one time, it can be added in multiple times, and the precipitate should be avoided when sucking up), at room temperature 12,000 rpm (≈ 13,000×g) Centrifuge for 1 min. Discard the waste liquid in the collection tube.

12. Add 600 μl of Wash Buffer solution (make sure to add absolute ethanol), centrifuge at 12,000 rpm (≈13,000×g) at room temperature for 1 min, and discard the waste solution.

13.Repeat step 12.

14. Centrifuge at 12,000 rpm (≈13,000×g) for 2 min at room temperature to completely shake off the Wash Buffer residue.

15. Take out the P column of the adsorption column and put it into a new EP tube. Open the cover of the adsorption column P and let it stand for 2 minutes at room temperature. If necessary, place it in the air-conditioning vent for 1-2 minutes to completely remove the residue of ethanol.

16. Add 100-200 μl (at least 100 μl dissolving volume) Elution Buffer or ddH_2O to the middle of the P column of the adsorption column (the effect is better after a 56 ℃ water bath), and let stand for 5 minutes to completely dissolve the adsorbed plasmid, Centrifuge at 13,000 ×g at room temperature for 2 min to obtain the extracted plasmid.

Note: The extracted plasmid DNA can be directly used for gene cloning, sequencing, enzyme digestion, library screening, in vitro transcription and translation, and cell transfection.

DNA concentration and purity

DNA concentration (μg/ml) = OD_{260} × 50× dilution factor, OD_{260}/OD_{280} is about 1.8-2.0

Attention

Plasmid copy number: When purifying medium and low copy plasmids, use 2 times the volume of bacterial solution, 2 times the Solution A, B, C2, and the same volume of Wash Buffer and Elution Buffer.

Transformed bacteria: If it is a bacteria frozen in glycerol at -80 ℃, please spread it on the plate first and then select a new single colony for cultivation. Do not directly take frozen strains for cultivation.

Frequently Questions&Answers

1. No plasmid is proposed or the concentration of plasmid is very low

A. Strain aging

Suggestion: For bacteria preserved in glycerol, it needs to be activated first. Coat or streak the strains, re-select single colonies for liquid culture, and perform initial shake activation on the strains, and cultivate the strains according to the ratio of 1:500. It is best not to culture the cells for more than 16 hours.

B. Plasmid loss

Suggestion: Some plasmids will be lost in the process of multiple subcultures. In addition, check whether the concentration of the selected antibiotic is correct.

C. Insufficient cracking

Suggestion: If you use more than the recommended amount of bacteria for plasmid preparation, it will result in insufficient cell lysis. The amount of bacteria can be appropriately reduced or the amount of various solutions can be increased accordingly. Please process the corresponding amount of bacteria according to the selected kit.

D. There is precipitation in Solution that has not dissolved

Suggestion: Solution B and Solution C2 will precipitate when the temperature is low. Please check whether there is precipitation before use. If there is precipitation, please incubate at 37 ℃ for a while, and use it after the solution is clear.

E. Absolute ethanol was not added to DNA Wash Buffer as required.

Suggestion: Add the required amount of absolute ethanol according to the user manual, and tighten the cap after use to prevent the ethanol from evaporating.

F. The pH value of the dissolving solution is incorrect

Suggestion: The optimum pH value for dissolving DNA from the column is between 7.0 and 8.5. If the pH of the dissolving solution exceeds this range, it will significantly affect the dissolution effect. Please use the Elution Buffer (pH 8.0, 10 mM) that comes with the kit. Tris-HCl) for dissolution. If ddH2O or other solutions are used for dissolution, please ensure that the pH is between 7.0 and 8.5.

G. Selection of dissolving volume and time

Suggestion: The dissolution volume will affect the final yield. The larger the dissolution volume, the higher the yield, but the concentration will decrease. Please use the recommended dissolution volume of the kit for dissolution to ensure the best yield and concentration. If a high concentration of plasmid is required, please reduce the dissolution volume.

In addition, if you want to harvest high-concentration and high-yield plasmids, you can perform a second solubilization.

Suggestion: After adding Elution Buffer, leave it at room temperature for 2~5 minutes, which is more conducive to dissolution.

2. Plasmid purity is not high

A. Protein pollution OD_{260}/ OD_{280}<1.8

Suggestion: Choose the recommended amount of bacteria, and carefully aspirate the supernatant after centrifugation. If the supernatant is mixed with suspended matter, centrifuge again to completely remove the protein.

B. RNA contamination OD_{260}/ OD_{280}>2.0

Suggestion: Check whether the delivered RNase A is completely added to Solution A. After adding RNase, Solution A/RNase should be stored at 4 ℃. If the storage time is too long or not stored correctly, please re-add RNase.

C. Genomic DNA contamination

Suggestion: After adding Solution B, gently invert and mix to avoid violent shaking and vortexing. The processing time for adding Solution B should not exceed 5 min.