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EndoFree Plasmid Miniprep Kit

Catalog No.	Specification	Storage/Shelf life
EP004-50T	50T	Room temperature/1 year
EP004-200T	200T	Room temperature/1 year

Introduction:

In this kit. the plasmid DNA was purified rapidly by the modified SDS alkaline lysis combined with the method of selective adsorption of DNA on DNA preparation membrane. It applies to extract up to 20ug of high purity plasmid DNA from 1-5 ml bacterial culture. It has been used in molecular biology experiments such as sequencing. in vitro transcription and translation. restriction endonuclease digestion and bacterial transformation.

Kit Components

Component	EP004-50T	EP004-200T
Solution A	15 ml	60 ml
Solution B	15 ml	60 ml
Solution C	10 ml	40 ml
Endo-Remove Buffer	1 vial	1 vial
Wash Buffer	60 ml	240 ml
Elution Buffer	4 ml	10 ml
RNase A	1 vial	1 vial
Adsorption column P column	50 set	200 set
User manual	1 copy	1 copy

Before starting

Solution A: Add RNase A to Solution A. please store Solution A at 4℃.

Solution B、C: Sealed preservation. If Solution C is not used for a long time after opening. please check the pH of Solution C to ensure pH ≤ 4.8. If the pH is too high. adjust with a small amount of acetic acid.

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Wash Buffer: Add the anhydrous ethanol into the Wash Buffer (labeled on the reagent bottle) before use.

Endo-Remove Buffer：Long-term preservation must be placed on 2-8 ℃

Operation steps

1. Inoculate the bacteria to 1-5 ml liquid medium and shake for 12-16 h at 37℃. At room temperature. 13000 g centrifugation for 1 min，collecting the bacteria and absorb the supernatant as much as possible.

Attention:

a. The residual liquid medium is liable to cause insufficient bacterial cell lysis. After the fifth step. the precipitate is loose after centrifugation. and the supernatant cannot be effectively absorbed.

b. The procedure of this user manual is applicable to the bacterial solution with a culture medium OD_{600} (bacteria density) between 2.0 and 3.0 after 12-16 h of culture on standard LB (Luria Bertani) medium. If an enriched medium. such as TB or 2×YT.please ensure that the OD_{600} does not exceed 3.0.

2. Add 250μl of Solution A and fully suspend the bacterial cells by vortexing.

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

3. Add 250μl of Solution B. and mix gently by inverting 5-10 times to mix well. The solution is viscous and clear.

Attention:Do not oscillate vigorously. This step does not exceed 5 minutes. If the time is too long. it will cause genomic DNA contamination or plasmid damage.If the solution is not clear and clarified. it indicates that the cell lysis is not sufficient. so.the amount of Solution B should be increased or the amount of bacteria should be reduced.

4. Add 250μl of Solution C and mix it up 5-10 times. White flocculent precipitation appears at this time.

5，Transfer the centrifuge tube to a high-speed centrifuge and centrifuge at 12.000 rpm (≈13.000 × g) for 10 min at room temperature (if the white precipitate floats in the supernatant. centrifuge again) and carefully absorb the supernatant after centrifugation.

6. Add 1/10 volume of Endo-Remove Buffer to the supernatant (for example. 500 µl for the supernatant and 50 µl of Endo-Remove Buffer).

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

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

9. Centrifuge at 12.000 rpm (≈13.000×g) for 5 min at room temperature. the solution will be delaminate and carefully pipet the supernatant into a new 1.5 ml EP tube.

Attention: that 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 6-8 times. and let stand for 1-2 min at room temperature.

11. Add the mixture obtained in the previous step to the P column of the adsorption column provided by the kit (if it cannot be added at one time. it can be added in multiple times). and centrifuge at 12.000 rpm (≈13.000×g) for 1 min at room temperature. Discard the waste liquid from the collection tube.

12. Add 600µl of Wash Buffer (**ensure that absolute ethanol has been added**). centrifuge at 12.000 rpm (≈13.000×g) for 1 min at room temperature. and discard the waste.

13. Centrifuge at 12.000 rpm (≈13.000×g) for 2 min at room temperature to completely remove the Wash Buffer residue.

14. Take out the adsorption column P column and put it into a new EP tube. The adsorption column P column was opened at room temperature for 2 minutes to completely remove the residual ethanol.

15. Add 30-100 µl (recommended 50 µl of dissolved volume) to the middle of the P column of the adsorption column. Elution Buffer or ddH_2O (better after 56 ° C water bath). let stand for 5 min to completely dissolve the plasmid to be adsorbed. 12.000 (≈13.000) ×g)rpm at room temperature for 2 min for extraction of plasmid.

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

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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 the medium and low copy plasmid. use 2 times the volume of the bacterial solution. 2 times the Solution A. B. C. the same volume of Wash Buffer and Elution Buffer.

Transforming bacteria: If it is -80℃ glycerol frozen bacteria. please apply the plate culture before re-selecting a new single colony for culture. Do not directly take frozen strains for cultivation.

Frequently Questions & Answers

1. No plasmid or low plasmid concentration

a. Strain aging

Recommendations: For glycerol-preserved strains. activation is required first.Coating or streaking bacteria， re-select a single colony for liquid culture, and activate the strain by initial shaking,strain cultivation at a ratio of 1:500.The secondary cultured cells should preferably not exceed 16 hours.

b. Plasmid loss

Recommendations: some plasmids may be lost during repeated subculture. and the concentration of antibiotics should be screened correctly.

c. Insufficient cell lysis

Recommendations:if more than the recommended amount of bacteria are used for plasmid preparation. it will lead to insufficient cleavage of the bacteria. The amount of bacteria can be appropriately reduced or the amount of various buffers can be increased accordingly. Please process the appropriate amount of bacteria according to the selected kit.

d. Precipitate in buffer is not dissolved

Recommendations: Solution B and Solution C will precipitate at low temperatures. Check for precipitate formation before use. If precipitation occurs. please incubate at 37℃ for a while and clarify the solution.

e. Anhydrous ethanol was not added to the DNA Wash Buffer as required.

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Recommendations:add the required amount of anhydrous ethanol according to the instructions. and screw the cap after use to prevent the ethanol from volatilization.

f. dissolved solution pH is incorrect

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

g. Solution volume and time selection

Recommendation:The dissolved volume will affect the final yield. The larger the dissolved volume. the higher the yield. but the lower the concentration. 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. reduce the volume of dissolution. In addition. if it is desired to harvest a high concentration and high yield of the plasmid. secondary dissolution can be performed.

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

2. Plasmid purity is not high

a. Protein contamination OD_{260}/ OD_{280}<1.8

Recommendation: Select the recommended amount of cells. carefully absorb the supernatant after centrifugation.If suspension is mixed in the supernatant. centrifuge again to completely remove the protein.

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

Recommendation: Check that the delivered RNase A is fully added to Solution A. After adding RNase. Solution A/RNase should be stored at 4 °C. If the storage time is too long or not stored properly. please re-add RNase.

c.Genomic DNA contamination

Recommendation: After adding Solution B. gently invert and mix to avoid violent vortex. It is better not to add Solution B for more than 5 minutes.