Gencyst

For research use only.

Plasmid Prepare Kit

Catalog No.	Specification	Storage/Shelf life
EP002-50T	50T	Room temperature/1 year
EP002-200T	200T	Room temperature/1 year

Introduction

In this kit. Combining the method of preparing membrane with high adsorption amount of DNA and selectively adsorbing DNA to achieve the purpose of rapid purification of plasmid DNA. It applies to extract up to 60ug of high purity plasmid DNA from 5-30 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	EP002-50T	EP002-200T
Solution A	25 ml	100 ml
Solution B	25 ml	100 ml
Solution C2	35 ml	140 ml
Wash Buffer	60 ml	240 ml
Elution Buffer	10 ml	30 ml
RNase A	1 vial	1 vial
Adsorption column P column	50 set	200 set
User Manual	1 copy	1 copy

Gencyst

For research use only.

Before starting

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

Solution B、C2: Sealed preservation. If Solution C2 is not used for a long time after opening. please check the pH of Solution C2 to ensure pH ≤ 4.8. If the pH is too high. adjust with a small amount of acetic acid.

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

Operation steps

1. Inoculate the bacteria to 5-30 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 500µl 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 500µl 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 700µl Solution C2 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 the supernatant obtained in the previous step to the P column of the adsorption column provided by the kit (if it can not be added once. it can be added in multiple times. avoiding the precipitation when sucking). The supernatant was centrifuged at room temperature for 1 min at 12.000 rpm (13.000 *g). Discard the waste liquid from the collecting pipe.

7. Add 600 µl of Wash Buffer solution (**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.

8. Repeat step 7.

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

10. Take out the adsorption column P column and put it into a new EP tube. Open the lid of the adsorption column P column and let it stand still for 2 min at room temperature. If necessary. put it in the air conditioning vent for 1-2 min to completely remove the residual ethanol.

11. Add 100-200µl (at least 100µl dissolved volume) Elution Buffer or ddH_2O to the middle of the P column of the adsorption column (the effect is better after 56℃ water bath). And let stand for 5 min for adsorption of plasmid completely dissolved. 13000 x g centrifugal 2 min at room temperature 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. If used to transfect endotoxin-sensitive cell lines.primary cells and microinjection. removal of endotoxin is recommended.

DNA concentration and purity

DNA concentration(µg/ml)=OD_{260} × 50×Dilution factor.

OD_{260}/ OD_{280} is about 1.8-2.0

Gencyst

For research use only.

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. C2. the same volume of Wash Buffer and Elution Buffer.

Transforming bacteria: If the bacteria are frozen in glycerol at -80 °C. 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 solutions can be increased accordingly. Please process the appropriate amount of bacteria according to the selected kit.

d. Precipitate in solution is not dissolved

Recommendations: Solution B and Solution C2 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.

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 ddH_2O 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 ℃. 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.