ELK Biotechnology

For research use only.

Max Plasmid Purification Kit

Catalog No.	Specification	Storage/Shelf life
EP003-10T	10T	Room temperature/1 year
EP003-20T	20T	Room temperature/1 year

Introduction:

This kit uses a unique buffer system that combines with traditional isopropanol precipitation to quickly obtain large amounts of high-purity plasmid DNA. The plasmid DNA extracted using this kit can be applied to various routine operations. Including restriction enzyme digestion, PCR, sequencing, ligation, transformation and transfection of a variety of cell experiments.It is recommended to use the amount of bacteria every time: the recommended amount of high-copy plasmid is 100 ml. the yield is generally about 200-1500ug the recommended amount of low-copy plasmid is 200 ml. and the yield is generally about 100-400ug.

Kit Components

Component	EP003-10T	EP003-20T	Storage
RNase A	250 µl	500 µl	-20℃
Solution A	50 ml	100 ml	RT
Solution B	50 ml	100 ml	RT
Solution C	50 ml	100 ml	RT
MagPurp	250 µl	500 µl	RT
Elution Buffer	10 ml	20 ml	RT
Filter column S column	10 vial	20 vial	RT
User manual	1 copy	1 copy	RT

Before starting

The kit can be stored under dry conditions at room temperature (15-25 ℃) for 12 months.and at 2-8 °C for longer periods of time. Under the storage conditions of 2-8 °C. if the solution is precipitated. the solution in the kit should be allowed to

stand at room temperature for a period of time before use. If necessary. preheat it in a 37°C water bath for 10 min to dissolve the precipitate.

a). Solution A**:** Add RNase A to Solution A, please store Solution A at 4℃.

b).Check if Solution B and C crystallize or precipitate before use. If there is crystallization or precipitation. it can be clarified by heating in a 37°C water bath for a few minutes.

c). Prepare about 500 ml of isopropanol and 75% ethanol.

d). Be careful not to touch Solution B and C directly. and cover the cover immediately after use.

e). When using the filter. pull the push handle carefully and slowly out of the filter tube to prevent the membrane from loosening due to pressure.

f). The amount of plasmid extracted is related to factors such as bacterial culture concentration and plasmid copy number. If the plasmid is a low-copy plasmid or a large plasmid larger than 10 kb. increase the amount of bacteria used. and increase the amount of A. B. and C proportionally; Elution Buffer is recommended to preheat in a 65-70 °C water bath.

g). How to use MagPurp: MagPurp is an indicator to indicate the correctness of the whole operation and is harmless to the human body. MagPurp is an optional reagent and customers can choose whether to add it according to their needs. If transfection experiments are required after plasmid extraction. it is not recommended to add MagPurp reagent to the experiment. When using. mix according to MagPurp:Solution A=1:200. thoroughly mix and invert. and the mixed solution is clear purple-red. Add the mixed solution to the collected cells and mix thoroughly. The mixed solution is turbid purple-red due to the presence of the cells. After the solution B is thoroughly mixed. the color of the solution is clear. Purple. indicating full lysis; after adding Solution C to thoroughly mix. the solution is yellow. indicating that the neutralization and renaturation are sufficient.

Operation steps

1. Take 100 ml (select the appropriate amount according to the concentration of cultured cells. 200 ml for low copy). Incubate the culture solution overnight. centrifuge at room temperature 10.000 rpm (~11.500×g) for 3 min. collect the bacteria. and absorb the supernatant as much as possible. To ensure that the

supernatant is completely absorbed. Please turn it upside down on clean absorbent paper.

Attention: when the bacterial fluid is more. it can be centrifuged many times and collect the bacteria cell pellet into a centrifuge tube.

2. Add 5 ml of Solution A to the centrifuge tube with the bacterial cell pellet (please ensure that RNase A has been added) and completely suspend the bacterial cell pellet using a pipette or vortex shaker.

Attention: Be sure to suspend thoroughly. If there are bacteria that are not thoroughly mixed. it will affect the lysis. resulting in low extraction and purity. The addition of the MagPurp reagent had no effect on subsequent PCR, enzyme Digestion and sequencing. When using. mix according to MagPurp:Solution A=1:200.Mix thoroughly upside down. and the mixed solution is purple-red. The mixed solution is added to the collected cells and thoroughly mixed. The solution after mixing is turbid purple due to the presence of the cells. If transfection experiments are required after plasmid extraction. it is not recommended to add MagPurp reagent to the experiment.

3. Add 5 ml of Solution B to the centrifuge tube and gently shake it up and down 6-8 times to allow the cells to be fully lysed and allowed to stand at room temperature for 5 min.

Attention: The movement is gentle. do not shake violently. so as not to pollute the genomic DNA. At this point. the bacterial liquid should become clear and viscous. If it does not become clear. the bacterial cells should be too much. and the lysis should be incomplete. The amount of bacteria should be reduced. If MagPurp is used. add Solution B and mix thoroughly. The color of the solution is clear purple. If there is a turbid purple red mixed in purple. the cracking is insufficient. and the mixing is continued until the color of the solution completely turns into a clear purple color.

4. Add 5 ml of Solution C to the centrifuge tube. immediately gently flip it up and down 6-8 times. mix well. until the solution appears white dispersion flocculent precipitate. Leave it at room temperature for about 10 minutes.
5. Centrifuge at 10.000 rpm (~11.500×g) for 10 min to remove the white precipitate to the bottom of the tube (increasing the centrifugation time). transfer to a new 50 ml centrifuge tube. and carefully pour all the solution into the S column. (Please avoid pouring a large amount of sediment and clogging the filter). slowly push the push handle to filter. and collect the filtrate in a clean 50 ml tube (customer-supplied).

Attention: Mix immediately after adding Solution C to avoid local precipitation. If there are too many cells (>100 ml). it is recommended to extend the centrifugation time to 20-30 min. If MagPurp is used. add Solution C and mix thoroughly. The solution is light yellow and the precipitate is yellow. If it is mixed with purple in yellow. it means that the renaturation is not enough. Continue to mix until the color of the solution becomes completely clear yellow.

6. Add 2/3 times the filtrate volume of isopropanol to the filtrate and mix thoroughly by inverting upside down.
7. Centrifuge at 10.000rpm (~11.500 × g) for 30 min at 4 ℃. gently pour off the supernatant and place it on absorbent paper.
8. Added 6 ml 75% ethanol to the tube. rinsed and precipitated thoroughly. centrifuge at 10.000 rpm (~11.500 × g) for 10 min at 4 ℃. gently pour off the supernatant and place it upside down on absorbent paper.
9. Repeat step 8.
10. Place the centrifugal tube open at room temperature for 10-20 minutes. allow the ethanol to fully evaporate. and add 0.3-1 ml (0.2-0.5 ml for low copy number) Elution Buffer to fully dissolve the precipitate.

Attention: The pH of the eluent has a large effect on the elution efficiency. If ddH_2O is used as the eluent. the pH should be within the range of 7.5-8.0. If the pH is lower than 7.0. the elution efficiency will be lowered. The amount of Elution Buffer used is mainly determined by the copy number of the plasmid and the concentration required for the experiment. DNA products should be stored at -20 °C to prevent DNA degradation.

DNA concentration and purity detection

The plasmid DNA can be detected for concentration and purity by agarose gel electrophoresis and ultraviolet spectrophotometry. Electrophoresis may be multi-band. which are caused by the multimer of the plasmid.and do not affect the subsequent enzyme digestion. transfection and the like. An OD_{260} value of 1 corresponds to approximately 50 ug/ml of double stranded DNA. The purified plasmid DNA OD_{260}/OD_{280} is usually about 1.8-2.0. and can be directly applied to conventional operations such as molecular biology. If buffer is not used and ddH_2O is used. the ratio will be low. but it does not mean that the purity is low because the pH and the presence of ions will affect the light absorption.

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. same volume of 70% ethanol and Elution Buffer.

Transforming bacteria: If the bacteria are frozen 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 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. 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 8.5, 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.

f. 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.