BonaPure Endotoxin-Free Maxi Plasmid Kit(Plus)

Cat. No.

PE118-10

Components

Components	Amount(10 rxns)
RNase A (10 mg/mL)	1.1 mL
Buffer BL	30 mL
Buffer P1	110 mL
Buffer P2	110 mL
Buffer P4	110 mL
Endotoxin Removal Buffer	32 mL
Buffer W1	75 mL
Elution Buffer	25 mL
Adsorption Column EC (with Collection Tubes)	10
Collection Tubes	10

Storage Condition

The kit can be stored in a dry environment at room temperature (10~25°C) for 15 months. The RNase A package alone can be stored at -20°C for 2 years and can be transported at room temperature. Buffer P1 after adding RNase A can be stored for 6 months at 2~8℃

Introduction

This kit uses an improved alkali lysis treatment method and silica membrane adsorption technology to obtain endotoxin-free high-purity plasmid DNA specifically and efficiently; the unique endotoxin precipitation technology requires no filtration and is easy to operate. The obtained plasmid can be directly used in cell transfection, enzyme digestion, ligation, transformation, PCR, sequencing and other molecular biology

experiments. For high-copy plasmids, 100 mL of bacterial fluid can usually obtain 500~1500 μg of plasmid. For low-copy plasmids, 200 mL of bacterial fluid can usually obtain 200~600 μg of plasmid.

Highlights

- High purity: Use unique endotoxin precipitation technology to specifically remove endotoxins
- High-efficiency transfection: suitable for transfection of most cells, including endotoxin-sensitive cells
- Easy to operate: Using adsorption column technology to specifically adsorb plasmids, no filtration is required, and the operation is easier
- Wide application: can be used in animal and plant cell transfection and molecular biology experiments

Scope of application

This product is suitable for extracting up to 1500 μg of high-purity endotoxin-free plasmid DNA from 100 mL~200 mL bacterial cultures.

Imporatant Notes

1. The bacterial culture time is generally 12 to 16 hours. If the inoculation amount is large, the culture time should be reduced. Over-culture will reduce the quality of the plasmid and even cause mutations;
2. Every time you use it, you need to observe whether Buffer P2 and Buffer P4 form a precipitate. If there is any precipitate, dissolve it at 37°C before use;
3. It is recommended to use the column treated with equilibrium solution immediately. Leaving it for too long will affect the use effect;
4. Before first use, add 175 mL of absolute ethanol to Buffer W1 according to the label on the bottle;
5. Add RNase A to Buffer P1 and store at 2~8℃;
6. Please close the cap tightly after using each solution;
7. All operations are performed at room temperature;
8. The yield and quality of plasmid extraction are related to the type of host bacteria, plasmid copy number, plasmid stability and other factors.

Protocol

Prepare your own: absolute ethanol, isopropyl alcohol, 50 mL centrifuge tube.

1. Equilibration: Add 2.5 mL of Buffer BL (processed on the same day) to the adsorption column (put the

adsorption column into the collection tube), centrifuge at 10,000 rpm for 2 minutes, discard the filtrate in the collection tube, and put the adsorption column back into the collection tube. in the tube;;

2. Take 100~200 mL (for high-copy plasmids, 100 mL of bacterial liquid is recommended; for low-copy plasmids, 200 mL of bacterial liquid is recommended, with a maximum of 300 mL of bacterial liquid) of the bacterial liquid cultured overnight, and centrifuge at 10,000 rpm for 2 minutes. Discard the supernatant;
3. Add 10 mL Buffer P1 (**add all the RNase A provided in the kit before use**), vortex or pipet fully with a pipette to resuspend the bacteria evenly;

Note: Ensure that the bacterial sediment is evenly suspended. Containing unsuspended bacterial clumps will affect lysis, resulting in a reduction in the concentration and purity of the extracted plasmid.

4. Add 10 mL Buffer P2, mix gently by inverting to completely lyse the bacteria;

Note: Do not shake vigorously to avoid contamination of genomic DNA; do not use it for more than 5 minutes to avoid damage to the plasmid. If it does not become completely clear, there may be too many bacteria. You can increase the amount of Buffer P2 in subsequent operations. Press the middle button to increase the amount of Buffer P4 in multiples.

5. Add 10 mL Buffer P4 and mix gently by inverting immediately until white floc appears in the solution. Centrifuge at 10,000 rpm for 10 minutes to allow the white precipitate to centrifuge to the bottom of the tube (the centrifugation time can be increased appropriately), and carefully transfer the supernatant to a clean centrifuge tube (prepared by yourself) **(do not bring the precipitate);**

Notice

1）Bu1) Buffer P4 should be mixed immediately after addition to avoid local precipitation;

2) After centrifugation, a dense floating film may form on the top layer. Be careful not to pour it into the adsorption column.

6. Add 3 mL Endotoxin Removal Buffer, invert and mix;

Note: Endotoxin Removal Buffer may be stratified, which does not affect use. Just shake well before use.

7. Add 0.3 times the volume of isopropyl alcohol to the above supernatant, and mix by inverting (**adding too much isopropyl alcohol can easily cause RNA contamination);**
8. Transfer the mixed solution obtained in step 7 to the balanced adsorption column EC (with Collection Tubes), centrifuge at 10,000 rpm for 1 min, and discard the filtrate in the collection tube **(the maximum volume of the adsorption column is 15 mL, which is the solution obtained in the previous step It needs to be passed through the column 2 to 3 times)**;

Note: If the centrifuge rotor has a large inclination angle, it is recommended that the volume of solution added to the adsorption column does not exceed 10 mL to prevent leakage

9. Add 10 mL Buffer W1 (check whether absolute ethanol has been added before use), centrifuge at 10,000 rpm for 1 min, and discard the filtrate;
10. Repeat step 9;
11. Put the adsorption column EC back into the empty collection tube, and centrifuge at 10,000 rpm for 5 minutes;
12. Place the adsorption column EC into a new 50 mL centrifuge tube, open the lid and leave it for 5 minutes to completely evaporate the ethanol;
13. Add 1~2 mL Elution Buffer to the middle part of the adsorption membrane (**60~65°C preheating effect is better)**, place it at room temperature for 2 minutes, and centrifuge at 10,000 rpm for 2 minutes to obtain plasmid DNA **(if a larger amount is needed) DNA, the resulting solution can be transferred back to the adsorption column and this step can be repeated).**

Q&A

Q1：Why is plasmid DNA yield low?

A1：

1) The plasmid copy number is low, the plasmid is >10 kb, or it is a Gram-positive bacterial plasmid. The amount of bacteria should be increased, 300~500 mL of overnight culture can be used, the eluent Elution Buffer should be preheated in a 60°C water bath, and the adsorption and elution time can be appropriately extended to increase the extraction efficiency;

2) Bacteria problem. There is a phenomenon of plasmid loss during the storage of bacterial strains. It is recommended to streak and activate the bacteria before cultivating them to stabilize the yield;

3) The bacteria are not fully lysed. Bacteria must be fully resuspended in Buffer P1/RNase A or there should not be too many bacteria to avoid clumping or excessive bacteria that cannot be lysed and reduce the yield.

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

A2：

1) The bacterial liquid culture time is too long. The bacterial culture time needs to be controlled within 12 to 16 hours;
2) Cracking problem. When adding Buffer P2, it must be gently inverted and mixed; when processing multiple samples, the total time from the time of adding Buffer P2 should not exceed 5 minutes.