# FastPure EndoFree Plasmid Maxi Kit

## DC202

Vazyme

Instruction for Use

Version 22.2

Vazyme

# Contents

## 01/Product Description

This kit is suitable for extraction from 150 - 300 ml of bacterial solution cultured overnight, using an improved SDS-alkaline lysis method to lyse the bacteria. The crude extract is selectively combined with a unique Endotoxin Scavenger and separated by centrifugation to remove endotoxins. Then, the silica gel membrane selectively binds to plasmid DNA in the solution under conditions of high-salt and low-pH. This is followed by addition of wash buffer to remove impurities and other bacterial components. Finally, a low-salt, high-pH elution buffer is used to elute pure plasmid DNA from the silicon matrix membrane. The silica gel membrane employs special adsorption membrane, and the adsorption amount difference between the column and the column is very small and the repeatability is good. Phenol, chloroform and other toxic reagents are not required, and neither is ethanol precipitation steps. This kit can be used to rapidly extract 0.2 - 1.5 mg of pure high-copy plasmid DNA, with an extraction rate of 80% - 90%. The kit uses an unique process formula removes endotoxin, the content of endotoxin is extremely low and the cell transfection effect is excellent. The extracted plasmid could be directly used in enzyme digestion, PCR, in vitro transcription, transformation, sequencing and other molecular biology experiments.

## 02/Components

| Components | DC202-01 (10 rxns) |
|---|---|
| RNase A | 750 µl |
| Buffer P1 | 75 ml |
| Buffer P2 | 75 ml |
| Buffer P4 | 75 ml |
| Endotoxin Scavenger | 25 ml |
| Buffer PW | 2 × 22 ml |
| Buffer TB | 20 ml |
| FastPure DNA Maxi Columns (each in a 50 ml Collection Tube) | 10 |
| Endotoxin-free Collection Tube | 2 × 5 |

RNase A: 10 mg/ml, used to remove RNA.
Buffer P1: bacterial suspension buffer, add RNase A to Buffer P1 before first use.
Buffer P2: bacterial lysis buffer (containing SDS/NaOH).
Buffer P4: neutralizing buffer.
Endotoxin Scavenger: effectively remove endotoxin from the crude plasmid extract.
Buffer PW: wash buffer, add the stipulated volume of ethanol before first use.
Buffer TB: elution buffer.
FastPure DNA Maxi Columns: plasmid DNA adsorption columns.
Collection Tubes 50 ml: filtrate collection tubes.
Endotoxin-free Collection Tube: plasmid DNA collection tubes.

## 03/Storage

RNase A should be stored at -30 ~ -15℃ and transported at ≤0℃.

Endotoxin Scavenger can be stored at 2 ~ 8℃ for one month, stored at -30 ~ -15℃ for long-term storage and transported at ≤0℃.

Other components should be stored at room temperature (15 ~ 25℃) and transported at room temperature.

## 04/Applications

This product is suitable for large-scale extraction of plasmids from 150 - 300 ml of bacterial solution cultured overnight.

## 05/Self-prepared Materials

Absolute ethanol, isopropanol, 50 ml round-bottom centrifuge tubes and 50 ml endotoxin-free centrifuge tubes.

## 06/Notes

For research use only. Not for use in diagnostic procedures.

1. Add all of the RNase A provided in the kit to Buffer P1 before use and store at 2 ~ 8℃.
2. Refer to the label on Buffer PW to add the corresponding volume of absolute ethanol before use. Mix well before use and store at room temperature.
3. The yield and quality of plasmid extraction are related to the type of host bacteria, culture concentration, plasmid copy number, plasmid stability and other factors. When processing low-copy plasmids or large plasmids (>10 kb), the volume of the bacterial solution should be increased and the volumes of Buffer P1, Buffer P2 and Buffer P4 used should be increased proportionally. Buffer TB should be preheated to 55℃ and the adsorption and elution times should be extended appropriately to improve elution efficiency.
4. Precipitation may occur in Buffer P2 and Buffer P4 at low temperature. These precipitates can be dissolved in a 37℃ water bath and these buffers can be used after cooling to room temperature.
5. Don't touch Buffer P2 and Buffer P4 directly. Wear disposable gloves when using these buffers and close the lid immediately after use.
6. Agarose gel electrophoresis and UV spectrophotometer can be used to measure the purity and concentration of the extracted plasmid DNA. The gel may show one, two or more bands. This is mainly due to differences in mobility of plasmids with different degrees of superhelix conformation and is related to the length of the culture time of the extract and the severity of operation during extraction. Under normal circumstances, the degree of superhelix in plasmids extracted using this kit is more than 90%.
7. The exact molecular size of the plasmid DNA must be determined after digestion and linearization, and comparison with the DNA Marker. The locations of circular or supercoiled plasmids have an indeterminate migration position, and the exact size can not be known by electrophoresis.

## 07/Mechanism & Workflow

Resuspension
Lysis
Neutralization

◇ Add 7.5 ml Buffer P1
◇ Add 7.5 ml Buffer P2
◇ Add 7.5 ml Buffer P4

Endotoxin removal

◇ Add 0.1 times the supernatant volume of Endotoxin Scavenger

Binding
Washing

◇ Add 0.5 times the volume of isopropanol
◇ Add 10 ml Buffer PW
◇ Add 10 ml Buffer PW

Elution

◇ Add 1 - 2 ml Buffer TB or Endotoxin-free dd$H_2O$

## 08/Experiment Process

1. Take 150 - 200 ml (no more than 300 ml) of bacterial solution cultured overnight and centrifuge at about 11,000 rpm (12,000 × g) for 1 - 2 min. Discard the supernatant and collect bacteria.
   ▲ When collecting more than 50 ml of bacterial solution, the bacteria can be collected by adding bacterial solution, centrifugation, discarding the supernatant and other steps in the same 50 ml tube for multiple times.
2. Add 7.5 ml of Buffer P1 (please check whether RNase A has been added to Buffer P1) to the centrifuge tube containing bacteria and mix thoroughly by vortex or pipetting.
   ▲ The complete resuspension of bacteria in this step is critical to yield, and there should be no bacterial clumps after resuspension. If there are bacterial clumps that are not thoroughly mixed, it will affect the lysis, resulting in low yield and purity. If the $OD_{600}$ of bacterial solution is 0.65, it is recommended that 7.5 ml of Buffer P1 be used when extracting from 150 ml of bacterial solution; when $OD_{600}$ is 0.75, 8 ml of Buffer P1 should be used and the volumes of Buffers P2 and P4 should be changed accordingly. If the volume of the bacterial solution is increased to 200 ml, it is recommended that the volume of Buffers P1, P2, and P4 be increased proportionally.

3. Add 7.5 ml of Buffer P2 to the bacterial suspension from step 2 and mix gently up and down for 6 - 8 times and incubate at room temperature for 4 - 5 min.
   ▲ Invert gently to mix thoroughly. Vortexing will cause the genomic DNA fragmentation, resulting in genomic DNA fragments in the extracted plasmid. At this time, the solution becomes viscous and translucent, showing that the bacteria have been fully lysed. The duration should not exceed 5 min to avoid destruction of the plasmids. If the solution is not clear, there may be too many bacteria resulting in incomplete lysis, so the amount of bacteria should be reduced appropriately.

4. Add 7.5 ml of Buffer P4 to the bacterial suspension from step 3 and immediately invert gently 6 - 8 times to allow the solution to completely neutralize Buffer P2. At this time, white flocculent precipitate should appear. Centrifuge at more than about 11,000 rpm (12,000 × g) for 10 - 15 min, carefully pippette the supernatant into a new 50 ml round-bottom centrifuge tube (self-prepared), and avoid aspirate the floating white precipitate.
   ▲ Add Buffer P4 and immediately invert to mix well. Leave the tube to stand until the white precipitate is distributed evenly throughout the solution to prevent the production of local precipitation which could affect neutralization. If there is no uniform white flocculent precipitate before centrifugation and the supernatant is not clear after centrifugation, the tube can be centrifuged for another 5 min.

5. Add 0.1 times the volume (10% of the supernatant volume, about 2.2 ml) of Endotoxin Scavenger to the supernatant from step 4 and invert to mix. Place the solution in an ice bath or insert into crushed ice (or refrigerator freezer) for 5 min until the solution changes from turbid to clear and transparent (or still slightly turbid), and occasionally mix several times.
   ▲ After the Endotoxin Scavenger is added to the supernatant, the supernatant will become turbid but the supernatant should become clear (or slightly turbid) after cooling in the ice bath.

6. After the supernatant is placed at room temperature (>25℃) for 10 - 15 min, it will become turbid as its temperature increases to room temperature. Then the supernatant should be inverted to mix.
   ▲ If room temperature is lower or you want to reduce extraction time, the supernatant can be incubated in a 37 ~ 42℃ water bath for 5 - 10 min and the next step can be carried out after the supernatant becomes turbid.

7. Centrifuge the supernatant at about 11,000 rpm (12,000 × g) for 10 min at room temperature (temperature must be >25℃) to separate the phase. The upper aqueous phase contains the DNA while the lower blue oily phase layer contains endotoxin and other impurities. Transfer the DNA-containing aqueous phase to a new tube and discard the oily layer.
   ▲ The temperature during centrifugation must be over 25℃ as effective phase separation does not occur if the temperature is too low.
   ▲ If the phase separation is not effective, the centrifugation temperature can be adjusted to 30℃ and the time of centrifugation can be increased to 15 min.
   ▲ Don't suck the blue oily layer as it contains endotoxin and other impurities.

8. Add 0.5 times the volume of isopropanol (about 10 ml) to the upper aqueous phase and invert sufficiently to mix. Divide the solution several times into the adsorption column (the adsorption column had been placed in the collection tube) and centrifuge at about 11,000 rpm (12,000 × g) for 1 min. Discard the filtrate in the collection tube. Repeat the step until all mixed solution passes through the adsorption column.
   ▲ The volume of solution passing through the column should not exceed 15 ml each time. If the centrifuge employs a fixed-angle rotor, it is recommended that the column volume not exceed 12 ml.

9. Add 10 ml of Buffer PW (check if absolute ethanol has been added in advance!) and centrifuge at about 11,000 rpm (12,000 × g) for 1 min. Discard the filtrate. Place the adsorption column back in the collection tube and add another 10 ml of Buffer PW, centrifuge at about 11,000 rpm (12,000 × g) for 1 min. Discard the filtrate.

10. Use pipette to suck residual ethanol off between the inner pressure ring and column wall. Place the adsorption column back in the collection tube and centrifuge at maximum speed (preferably greater than about 11,000 rpm (12,000 × g)) for 3 min to dry the residual ethanol on silica gel membrane, then open the lid and dry at room temperature for 3 - 5 min.
    ▲ Residual ethanol will affect downstream enzyme reactions, such as enzyme digestion, enzyme ligation, PCR, etc. Don't omit this step.

11. Remove the adsorption column and place in a Endotoxin-free Collection Tube (provided with kit). Add 1 - 2 ml of Buffer TB (preheating Buffer TB in a 55℃ water bath can increase the yield) in the middle of the adsorption membrane, and place at room temperature for 3 min, then centrifuge at about 11,000 rpm (12,000 × g) for 3 min to elute the plasmids.
    ▲ In order to increase the efficiency of plasmid recovery, the solution obtained can be added to the adsorption column again and placed at room temperature for 3 min before centrifugation at about 11,000 rpm (12,000 × g) for 3 min. Performing elution twice can increase concentration by about 10%. The higher the elution volume, the greater the elution efficiency. If the required plasmid concentration is higher, the elution volume can be decreased (the volume should be no less than 1 ml). However, it should be noted that a low elution volume will decrease plasmid elution efficiency and plasmid yield. Increasing rotation speed will increase plasmid elution concentration; however, if the rotation speed is more than about 11,000 rpm (12,000 × g), the adsorption column may be trapped in the outer tube sleeve. During the experiment, the centrifuge tube that is compatible with the yellow adsorption column should be selected.

## 09/FAQ & Troubleshooting

| FAQ | Reasons | Solutions |
|---|---|---|
| Low DNA yield | 1. Low-copy plasmids | Differences in copy number will result in significant fluctuations in plasmid yield.<br>▲Low-copy plasmids: pBR322, pACYC and derivatives, pSC101 and derivatives, SuperCos, pWE15.<br>▲High-copy plasmids: pTZ, pUC, pBS and pGM-T.<br>The amount of bacteria should be increased for low-copy plasmids. 200 - 300 ml of bacterial solution cultured overnight should be used and the volumes of Buffer P1, Buffer P2, and Buffer P4 should be increased proportionally. Elution (Buffer TB) should be preheated in a 55℃ water bath. Additionally, the time of adsorption and elution can be extended to increase extraction efficiency. The other steps remain the same. |
| | 2. Large plasmids (>10 kb) | The amount of bacteria should be increased and 200 - 300 ml of bacterial solution cultured overnight should be used. The volumes of Buffer P1, Buffer P2, and Buffer P4 should be increased proportionally and the elution buffer (Buffer TB) should be preheated in a 55℃ water bath. Additionally, the time of adsorption and elution can be extended to increase extraction efficiency. The other steps remain the same. |
| | 3. Problems of strain | There is phenomenon of plasmid loss during the preservation of the bacteria. It is best to streak or spread the plate to activate bacteria before culturing bacteria to stabilize the yield. |
| | 4. Incomplete bacterial lysis | Bacteria must be thoroughly resuspended in Buffer P1/RNase A as bacterial clumps can't be lysed, which will decrease yield. |
| | 5. Incorrect reagent preparation | Buffer P2 will precipitate at low temperature and the solution must be heated to dissolve or placed in a 37℃ thermostat until the buffer is clear before use. The volume of ethanol added to Buffer PW isn't accurate (ethanol concentration should be controlled at 80%). |
| Genome contamination | 1. Culture time is too long | The culture time of bacterial solution should be controlled within 12 - 16 h. |
| | 2. Lysis problems | When adding Buffer P2, it must be gently reversed and mixed; When processing multiple samples, the total time should not exceed 5 min from the time of adding the first tube of Buffer P2. |
| Unsatisfactory downstream results | 1. Salt contamination | Make sure to wash twice with Buffer PW. |
| | 2. Ethanol contamination | After the final wash with Buffer PW, the centrifugation time of the adsorption column can be increased from the original 3 min to 5 min. |
| | 3. Shedding of silica membrane | During plasmid elution, the silica membrane may shed during centrifugation. It is recommended that the eluted DNA solution be re-centrifuged at about 11,000 rpm (12,000 × g) for 1 min. Carefully collect the supernatant. |
| RNA residue | 1. Activity of RNase A may decreased | Storage of Buffer P1 with added RNase A at 4℃ for more than 3 months may cause enzyme activity to decrease. When it is reused, add 10 mg/ml of RNase A to achieve a final concentration of 100 μg/ml in Buffer P1. |
| | 2.The addition of Buffer P1 is small | High concentration of bacterial suspension results in insufficient RNase A in Buffer P1 to digest the bacterial RNA. |

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