TIANGEN®

EndoFree Maxi Plasmid Kit V2

For purification of ultrapure plasmid with high yield from 100-200 ml overnight bacteria culture

www.tiangen.com/en

EndoFree Maxi Plasmid Kit V2

(Spin Column)

Cat.no. GDP120

Kit Contents

Contents	GDP120-01 (10 preps)
Buffer BL	30 ml
Buffer P1	125 ml
Buffer P2	125 ml
Buffer P4	125 ml
Endotoxin Removal Buffer ER	32 ml
Buffer ED	220 ml
Buffer PW	50 ml
Buffer TB	30 ml
RNase A (10 mg/ml)	1.25 ml
Filtration CS1	10
Spin Columns CP6	10
Collection Tubes (50 ml)	20
Handbook	1

Storage

EndoFree Maxi Plasmid Kit V2 can be stored dry at room temperature (15-30°C) for up to 15 months without showing any reduction in performance and quality. If any precipitate forms in the buffers, it should be dissolved by warming the buffers to 37°C before use. RNase A (10 mg/ml) can be stored for 15 months at room temperature (15-30°C). After adding RNase A, Buffer P1 is stable for 6 months at 2-8°C.

Introduction

The EndoFree Maxi Plasmid Kit V2 adopts a unique silica membrane adsorption technique to efficiently and specifically bind plasmid DNA. By combining the special Endotoxin Removal Buffer ER and Filtration CS1, endotoxin, proteins and other impurities can be effectively removed. The whole extraction process takes only 1 hour, ensuring the convenient and quick operation. The plasmid DNA extracted by this kit can be applied to various routine operations, including enzymatic digestion, PCR, sequencing, ligation, transformation as well as the transfection for various cell types.

Recommended bacteria amount for each practice: For high-copy plasmid, it is recommended to use 100 ml of bacteria culture media, and the yield from which is generally around 500-1500 μg. For low-copy plasmid, 200 ml bacteria culture media is recommended to generate around 50-300 μg plasmids.

Yield For Reference

Plasmid type	Bacterial Cells Volume	Plasmid Yield	Plasmid
Low copy	200 ml	50-300 μg	pBR322, pACYC, pSC101 SuperCos, pWE15
High copy	100 ml	500-1500 μg	pTZ, pUC, pBS, pGM-T

Important Notes Please carefully read before using this kit.

1. RNase A should be added into Buffer P1 before use **(add all RNase A provided in the kit)**. Mix well and store at 2-8°C.
2. Check if there's crystal or precipitate formed in Buffer BL, Buffer P2 or Buffer P4 before use. If crystal or precipitate are present, warm the solution in a 37°C water bath for a few minutes until the buffers turn clean.
3. Be careful not to touch Buffer P2 and P4 directly, and tighten the cap immediately after use.
4. When using the Filtration CS1, carefully pull the plunger out of the filter tube to avoid loosening the filter membrane due to pressure.
5. The amount of the plasmid extracted is related to factors such as bacterial culture concentration and plasmid copy number. If the plasmid is low-copy or is with the size larger than 10 kb, increase the bacterial culture amount, and the amount of Buffer P1, P2 and P4 should be increased in proportion. It is recommended to preheat the Buffer TB

in a 65-70°C water bath before use. The adsorption and elution time can be appropriately extended to improve the extraction efficiency.

6. Prior to each experiment, please balance the spin column with Buffer BL to maximize the activation of the silicon matrix membrane and increase the yield.
7. The column balanced with Buffer BL should be used immediately, otherwise the effect will be affected.
8. There may be slight color changes in Buffer ER after repeated uncapping, which does not affect the final plasmid extraction efficiency and purity.

Reagents need to be prepared by Customer

96-100% ethanol and isopropanol

Protocol

Please add the ethanol (96-100%) into Buffer PW before use. Please refer to the label on the bottle for the ethanol volume.

1. Column equilibration: Add 2.5 ml Buffer BL to the Spin Column CP6 **(place the Spin Column in a 50 ml Collection Tube)**. Centrifuge at 8,000 rpm (~8,228 × g) for 2 min. Dispose the waste liquid in the collection tube, and put the spin column back in the collection tube **(the column treated with Buffer BL should be used immediately)**.
2. Transfer 100 ml overnight cultured bacteria media into a centrifuge tube **(choose the proper amount according to the concentration of the cultured cells. For low-copy plasmids, 200 ml bacteria culture is recommended).** Collect the bacteria by centrifuging at 8,000 rpm (~8,228 × g) for 3 min at room temperature. Dispose the supernatant as much as possible.
   Note 1: For large amount of bacteria culture, collect the bacteria pellet into one centrifuge tube by splitting the bacteria culture into several centrifugation steps. Choose the amount of bacteria that can be fully lysed, because excessive bacteria will lead to insufficient lysis and reduce the extraction efficiency of the plasmid. If the plasmid is low-copy or is with the size larger than 10 kb, increase the bacterial culture amount, and the amount of Buffer P1, P2 and P4 should be increased in proportion.
   Note 2: For as low as 50 ml overnight cultured high copy bacteria media or 100 ml low copy one , the amount of Buffer P1, P2 and P4 should be decreased in proportion.
3. Remove the supernatant as much as possible. To ensure that the supernatant is completely removed, use a clean paper tissue to absorb the water droplets on

the bottle well.

4. Add 10 ml of Buffer P1 **(please first check if RNase A has been added)** to the centrifuge tube with bacteria pellet. Completely suspend the bacteria cell pellet by pipetting or vortexing.
   Note: Please ensure the complete suspension of the bacteria pellet. If the bacteria pellet is not thoroughly mix, the lysis efficiency will be affected, resulting in low extraction amount and low purity. For low-copy plasmids, increase the bacterial culture amount, and the amount of Buffer P1, P2 and P4 should be increased in proportion.
5. Add 10 ml of Buffer P2 to the centrifuge tube, and gently invert the tube 6-8 times to fully lyse the cells. Incubate at room temperature for 5 min.
   Note: Mix gently, do not vigorously oscillate, as this will cause contamination of genomic DNA. After this step, the bacteria suspension should become clean and viscous, if not, it might be due to excessive bacteria cells and incomplete lysis, which can be avoided by reducing the bacteria amount.
6. Add 10 ml of Buffer P4 to the lysate from step 5, and gently invert the tube 6-8 times to fully lyse the cells till white dispersion flocculent precipitate appears. Incubate at room temperature for 10 min. Centrifuge at 8,000 rpm (~8,228 × g) for 5-10 min until the precipitates are fully collected to the bottom of the tube **(the centrifuge time can be extended accordingly)**. Carefully pour all the supernatant in to Filtration CS1 **(please avoid pouring the precipitant in for they will block the filter)**. Gently push the plunger and filter the cell lysate into a clean 50 ml tube (self-prepared).
   Note: Mix the lysate immediately after adding Buffer P4 to avoid local precipitation. The filtration will not be affected if the lysate poured into Filtration CS1 contain the white precipitate. If large amount of bacteria is applied (> 100 ml), it is recommended to extend the centrifugation time to 20-30 min.
7. Add 3 ml of Endotoxin Removal Buffer ER, and mix by inverting the tube until the solution turns to a uniform transparent yellow color.
   Optional step: If lower endotoxin level is required, incubate the thoroughly mix solution above in an ice bath for 20 min, then incubate in a 42°C water bath for 5 min. Centrifuge at 5,000 rpm for 3 min and transfer the supernatant into a new 50 ml centrifuge tube.
8. For high-copy plasmids, add isopropanol with 0.3 × the volume of the above filtrate to the eluted DNA, and for low-copy plasmid, add 0.2 × isopropanol **(excessive isopropanol might easily cause RNA**

contamination). Mix by converting up and down and transfer the solution to the Spin Column CP6 **(keep the spin column in a 50 ml collection tube)**.
Note: Because the maximum volume of the Spin Column CP6 is 15 ml, so the solution needs to be split into two-time loading in the Spin Column. In some cases, the centrifuge rotor angle is too big, in this case, it is recommended to add less than 10 ml solution to the Spin Column to prevent leakage.

9. Centrifuge at 8,000 rpm (~8,228 × g) for 2 min at room temperature and dispose the waste liquid in the collection tube, then place the Spin Column CP6 back in the collection tube.
   Note: Please divide the solution obtained from step 8 to the spin column into two-time loading, and perform the operation according to the above conditions for each time.
10. Add 10 ml of Buffer ED to Spin Column CP6. Centrifuge at 8,000 rpm (~8,228 × g) for 2 min and dispose the waste liquid in the collection tube, then place the spin column back in the collection tube.
11. For low-copy plasmids, repeat step 10 once.
12. Add 10 ml of Buffer PW **(please check if ethanol has been added)** to the Spin Column CP6. Centrifuge at 8,000 rpm (~8,228 × g) for 2 min and dispose the waste liquid in the collection tube, then place the spin column back in the collection tube.
13. Repeat step 12 once.
14. Centrifuge at 8,000 rpm (~8,228 × g) for 5 min. Open the cap of the Spin Column CP6 and air-dry at room temperature for several minutes to thoroughly dry the residual Buffer PW in the absorbent membrane.
    Note: The residual ethanol in the Buffer PW will affect subsequent enzymatic reactions (enzymatic digestion, PCR, etc.).
15. Place the Spin Column CP6 in a clean 50 ml collection tube, and add 1-2 ml of Buffer TB to the middle of the adsorption membrane. Incubate at room temperature for 5 min and then centrifuge at 8,000 rpm (~8,228 × g) for 5 min at room temperature. Transfer the eluate from the 50 ml collection tube to a clean 1.5 ml centrifuge tube and store at -20°C.
    Note: In order to increase the recovery rate of plasmid DNA, it is optional to repeat step 15. It is recommended to preheat the Buffer TB in a 65-70°C water bath before use.The pH value of the elution buffer has a significant effect on the elution efficiency, so if ddH_2O is used for elution, please make sure the pH value is within the range of 7.5-8.0. if the pH value is lower than 7.0, the elution efficiency will be decreased. The volume of the elution buffer depends on the plasmid copy number and the concentration

of plasmids required for the experiment. The volume of the elution buffer should not be less than 1 ml, otherwise the elution efficiency will be affected.

Detection of Plasmid DNA Concentration and Purity

The purity and concentration of the extracted plasmid DNA can be detected by agarose gel electrophoresis and an ultraviolet spectrophotometer. An OD_{260} value of 1 corresponds to approximately 50 μg/ml of double stranded DNA. The OD_{260}/OD_{280} value of the purified plasmid DNA is generally in the range of 1.8-2.0. The purified plasmid DNA can be directly applied to experiments with high purity requirements, such as cell transfection or *in vivo* experiments.