TIANGEN®

EndoFree Midi Plasmid Kit

For purification of ultrapure plasmid DNA with high yield

www.tiangen.com/en

EndoFree Midi Plasmid Kit

Cat. no. 4992853

Kit Contents

Contents	4992853 10 preps
Buffer BL	30 ml
Buffer P1	30 ml
Buffer P2	30 ml
Buffer E3	15 ml
Buffer EBT	70 ml
Buffer GDE	30 ml
Buffer MRDE	36 ml
Buffer PWF	16 ml
Buffer TB	15 ml
RNase A (100 mg/ml)	150 µl
Filtration CS1	10
Spin Columns CP7	10
Collection Tubes 15 ml	20
Handbook	1

Storage Condition

After the addition of RNase A, Buffer P1 should be stored at 2-8°C and it would be stable for 6 months. RNase A can be stored for one year at room temperature (15-25°C). EndoFree Midi Plasmid Kit can be stored dry at room temperature (15-25°C) for up to 12 months. For longer storage, it should be stored at 2-8°C. If any precipitate forms in the buffers after storage at 2-8°C, it should be dissolved by warming the buffers at 37°C for several minutes before use.

Introduction

EndoFree Midi Plasmid Kit uses unique silica membrane technology which can specifically adsorb plasmid DNA efficiently. Meanwhile, this kit also uses unique Buffer EBT and Filtration CS1 to get rid of contaminants like endotoxin and protein compounds effectively. The whole experimental procedure of plasmid DNA extraction could be finished within 1 h. Plasmid DNA prepared by EndoFree Midi Plasmid Kit is suitable for a variety of downstream applications including restriction enzyme digestion, PCR, sequencing, ligation, transformation and cell transfection.

Cell culture volume: For the isolation of high copy plasmid DNA, 50 ml of *E. coli* cell culture is recommended, and the yield would be within 250-750 μg. For the isolation of low copy plasmid DNA, 100 ml of *E. coli* cell culture is recommended, and the yield would be within 100-300 μg.

Important Notes Before Starting

1. Add the provided RNase A solution to Buffer P1 (use 1 vial RNase A per bottle Buffer P1), mix, and store at 2-8°C.
2. Add ethanol (96-100%) to Buffer PWF and Buffer MRDE before use, check bottle tag for volume.
3. Check Buffer BL, P2, E3 and EBT before use to see if there is any precipitate formed. If necessary, dissolve the precipitate by warming at 37°C for several minutes.
4. Avoid direct contact of Buffer P2, E3 and EBT, immediately close the lid after use.
5. Draw out the plunger from the Filtration CS1 slowly to avoid membrane loose.
6. The plasmid yield is related to cell concentration and copy number of plasmid. If working with low copy vectors or large plasmid (>10 kb), it may be beneficial to increase culture volume and to increase Buffer P1, P2, E3 and EBT in proportion. Warm the Buffer TB at 65-70°C before use **(Prolong adsorption and elution time properly could increase extraction efficiency).**
7. Use Buffer BL to equilibrate spin columns before use could maximally activate silica membrane and increase the yield.
8. After treatment with Buffer BL, use the spin column as soon as possible, or else the efficiency would be reduced.

Protocol

Please add ethanol (96-100%) to Buffer PWF and Buffer MRDE before use, check the bottle tag for volume.

1. Column equilibration: place a Spin Column CP7 into a 15 ml collection tube and add 2 ml Buffer BL to Spin Column CP7. Centrifuge for 2 min at 5,000 rpm (~4,500 × g). Discard the flow-through, and place Spin Column CP7 back into the same collection tube **(use the Spin Column as soon as possible after treated with Buffer BL).**
2. Transfer 20-50 ml **(the specific volume depends on the cell concentration and copy number of plasmid, for low-copy plasmid, 100 ml is recommended)** overnight bacterial culture into a centrifuge tube and harvest cells by centrifuge at 5,000 rpm (~4,500 × g) for 3 min, and then remove all the supernatant by pipetting.

   Note: For large volume of bacterial cells, please harvest to one tube by several centrifugation steps. Too much bacterial cells will lead to incomplete lysis and further negative effect on plasmid yield.
3. In order to remove all the supernatant, please use absorption paper to dry the tube wall.
4. Resuspend pelleted bacterial cells in 2.5 ml Buffer P1 **(Ensure that RNase A has been added),** mix by vortex or pipetting.

   Note: No cell clumps should be visible after cell resuspension, since incomplete resuspension will reduce the yield and purity of isolated plasmid DNA. For low-copy plasmid, please increase the volume of cell culture and Buffer P1, P2, E3 and EBT in proportion.
5. Add 2.5 ml Buffer P2 and mix thoroughly by gently inverting the tube 6-8 times then place the tube at room temperature (15-25°C) for 5 min.

   Note: Mix by gently inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. After this step, the solution should become viscous and clear. If the solution won't turn clear, please reduce the amount of cells.
6. Add 1.25 ml Buffer E3, and mix immediately and thoroughly by gently inverting 6-8 times, white precipitate should be formed in solution at this point. Incubate the solution at room temperature (15-25°C) for 2-3 min, centrifuge at 5,000 rpm (~4,500 × g) for 10 min, precipitate should be concentrated at the tube bottom **(could prolong centrifugation time properly)**. Transfer all the supernatant to Filtration Column CS1 **(avoid**

transferring large clump into the Filtration CS1, which will clog the filtration membrane). Gently insert the plunger into the Filtration CS1 and filter the cell lysate into a new 50 ml tube (not supplied in the kit).

Note: To avoid localized precipitation, mix the lysate thoroughly and immediately after addition of Buffer E3. Small white precipitate in the supernatant won't have negative effect on filtration. If the cell culture volume is higher than 50 ml, please increase the centrifuge time to 20-30 min.

7. Add equal volume Buffer EBT to the cleared filtrate, mix completely by inverting 7-10 times.
8. Transfer 4 ml solution from step 7 to the Spin Column CP7 **(Place the Spin Column CP7 in a 15 ml collection tube).** Centrifuge at 5,000 rpm (~4,500 × g) for 3 min under room temperature (15-25°C). Discard flow-through and place the Spin Column CP7 back into the same collection tube. Repeat this step until all the solution from step 7 is treated.
9. Add 2 ml Buffer GDE to the Spin Column CP7 and centrifuge at 5,000 rpm (~4,500 × g) for 3 min. Discard the flow-through and place the Spin Column CP7 back into the same collection tube.
10. Add 3 ml Buffer MRDE **(Ensure that ethanol (96-100%) has been added)** to the Spin Column CP7 and centrifuge at 5,000 rpm (~4,500 × g) for 3 min. Discard the flow-through and place the Spin Column CP7 back into the same collection tube.
11. Add 3.5 ml Buffer PWF **(Ensure that ethanol (96-100%) has been added)** to the Spin Column CP7 and centrifuge at 5,000 rpm (~4,500 × g) for 3 min. Discard the flow-through and place the Spin Column CP7 back into the same collection tube.
12. Repeat step 11.
13. Centrifuge at 5,000 rpm (~4,500 × g) for 10 min to completely remove the residual buffer in the Spin Column CP7.

    Note: Residual ethanol will influence the subsequent enzymatic reactions (like enzyme digestion and PCR). In order to avoid the side effect of residual ethanol, please place the Spin Column CP7 with lid open under room temperature (15-25°C) for several minutes to dry the membrane.

14. Place the Spin Column CP7 in a clean 15 ml collection tube and add 0.5-1 ml Buffer TB to the center of the membrane and incubate 2-3 min at room temperature (15-25°C), centrifuge at 5,000 rpm (~4,500 × g) for 5 min. Transfer all the eluted plasmid solution to a 1.5 ml microcentrifuge tube and store at -20°C.

    Note: Transfer the plasmid solution back to the column and repeat step 14 would increase plasmid recovery efficiency. The pH value of elution buffer has significant influence on eluting; if distilled water is used to elute DNA, the pH value should be within 7.0-8.5. Low pH value (pH<7) would reduce the efficiency of eluting. The volume of elution buffer depends on the copy number of plasmid and the downstream experiment. Elution buffer should not be less than 0.5 ml, or else it may affect recovery efficiency. Extracted plasmid DNA should be stored at -20°C to avoid degradation

Measurement of DNA concentration and purity

The yield and purity of isolated plasmid DNA could be analyzed by agarose gel electrophoresis and UV spectrophotometry. An OD_{260} of 1 corresponds to a 50μg/ml dsDNA solution.

$OD_{260}/_{280}$ ratio value should be within 1.7-1.9, which suggests that the plasmid DNA can be used on experiments which have high requirement on plasmid DNA quality like cell transfection and even animal *in vivo* experiments.