TIANGEN®

EndoFree Maxi Plasmid Kit

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

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EndoFree Maxi Plasmid Kit

(Spin Column)

Cat.no. GDP117

Kit Contents

Contents	GDP117 10 preps
RNase A (100 mg/ml)	500 µl
Buffer BL	30 ml
Buffer P1	100 ml
Buffer P2	100 ml
Buffer P4	100 ml
Buffer PW	70 ml
Buffer TB	30 ml
Filtration CS1	10
Spin Columns CP6	10
Collection Tubes 50 ml	20
Handbook	1

Storage

EndoFree Maxi Plasmid Kit 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 (100 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

EndoFree Maxi Plasmid Kit is based on alkaline lysis technology followed by adsorption of DNA onto silica membrane in the presence of high salt, and uses Buffer P4 and Filtration CS1 to wipe off the endotoxin and protein impurity effectively. Plasmid DNA prepared by EndoFree Plasmid Kit is suitable for a variety of routine applications including restriction enzyme digestion, sequencing, library screening, ligation and transformation, in vitro translation, and transfection of robust cells.

Recommended bacterial culture volume: 500-1500 μg plasmid with 100 ml bacterial culture for high-copy vectors; and 200-600 μg plasmid with 200 ml bacterial culture for low-copy vectors.

Yield For Reference

Plasmid type	Bacterial Cells Volume	Plasmid Yield	Plasmid
Low copy	200 ml	200-600 μg	pBR322, pACYC, pSC101 SuperCos, pWE15
High copy	100 ml	500-1500 μg	pTZ, pUC, pBS, pGM-T

Important Notes Please read before use.

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. Check Buffer BL, P2 and P4 before use for salt precipitation. If necessary, dissolve the buffer by warming at 37°C.
3. Avoid direct contact of Buffer P2 and P4, immediately close the lid after use.
4. Draw out the plunger from the Filtration CS1 slowly to avoid membrane loose.
5. The amount of extracted plasmid is related to cells concentration and plasmid copy. 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, and P4 in proportion. Warm the Buffer TB to 65-70°C before use. **Prolong adsorption and elution time properly to increase extraction efficiency.**
6. Use Buffer BL to treat spin columns could activate silica membrane at maximum degree and higher yield.

7. After treated with Buffer BL, use the spin columns soon, since long-term placement may affect the purification effect.

Reagents need to be prepared by Customer

96-100% ethanol, isopropanol, 5M NaCl (optional) and 70% ethanol (optional).

Protocol

Add ethanol (96-100%) to Buffer PW before use, check bottle tag for the adding volume.

1. Column equilibration: place a Spin Column CP6 into a 50 ml Collection Tube (supplied in the kit) and add 2.5 ml Buffer BL to Spin Column CP6. Centrifuge for 2 min at 8,000 rpm (~8,228 × g). Discard the flow-through, and place Spin Column CP6 into the same Collection Tube **(use the spin columns as soon as possible after treated with Buffer BL).**
2. Harvest 100 ml (for low copy plasmid, please harvest 200 ml) overnight cultured bacterial cells by centrifuging at 8,000 rpm (~8,228 × g) for 3 min at room temperature (15-30°C), and then remove all traces of supernatant.

   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 reduce plasmid yield.
3. Try to remove all traces of supernatant, use clean paper tissue to absorb the fluids inside the tube wall.
4. Resuspend pelleted bacterial cells in 8 ml Buffer P1 **(Ensure that RNase A has been added)**. The bacteria should be resuspended completely by vortex or pipetting up and down until no cell clumps remain.

   Note: No cell clumps should be visible after re-suspension of the pellet, otherwise incomplete lysis will lead to lower yield and purity. For low copy plasmid, please increase Buffer P1, P2 and P4 volume too when increase bacterial cell volume.
5. Add 8 ml Buffer P2 and mix thoroughly by inverting the tube 6-8 times, then incubate at room temperature for 5 min.

   Note: Mix by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube

until the solution becomes viscous and clear. If the solution won't turn clear, please reduce the amount of cells.

6. Add 8 ml Buffer P4, and mix immediately and thoroughly by gently inverting 6-8 times, until the whole solution become cloudy. Incubate at room temperature for 10 min. Centrifuge for 5-10 min at 8,000 rpm (~8,228 × g), the white material should be in the bottom of the centrifuge tube **(prolong centrifugation time properly)**. Transfer the supernatant into a Filtration 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 P4. It will not affect filtration if there is small white precipitate in the supernatant that transferred to Filtration CS1. If using more than 100 ml bacterial culture, prolong centrifugal time to 20-30 min.

7. Add 0.3×volume isopropanol to the cleared lysate (too much isopropanol will lead to RNA contamination), mix completely by reverting upside and down and then transfer all solution to the Spin Column CP6 **(put Spin Column CP6 into 50 ml Collection Tube).**

   Note: The filtrate volume will loss after filtration, so added volume of isopropanol should be determined according to real filtrate volume. It is recommended to apply no more than 10 ml of filtrate to Spin Column CP6. For larger volumes, please divide the filtrate and centrifuge for several times.

8. Centrifuge for 2 min at 8,000 rpm (~8,228 × g). Discard the flow-through and place the Spin Column CP6 back into the same Collection Tube.

   Note: Centrifuge for two times under the above condition.

9. Add 10 ml Buffer PW **(Ensure ethanol has been added before use)** to the Spin Column CP6 and centrifuge at 8,000 rpm (~8,228 × g) for 2 min. Discard the flow-through and place the Spin Column CP6 back into the same Collection Tube.

10. Repeat step 9.

11. Add 3 ml 100% ethanol to the Spin Column CP6 (put the CP6 in a Collection Tube). Centrifuge for 2 min at 8,000 rpm (~8,228 × g), discard the flow-through.

12. Put Spin Column CP6 back to Collection Tube, centrifuge at 8,000 rpm (~8,228 × g) for 5 min for removing residual ethanol.

    Note: We suggest opening Spin Column CP6 lid and stay at room temperature for 2-5 min to completely dry the membrane.

13. To elute DNA, place the Spin Column CP6 in a clean 50 ml Collection Tube (supplied in the kit) and add 1-2 ml Buffer TB to the center of the membrane and incubate 5 min at room temperature, centrifuge at 8,000 rpm (~8,228 × g) for 2 min. Transfer the eluate from 50 ml centrifuge tube to a clean 1.5 ml centrifuge tube and put at -20 °C for storage.

    Note: Repeat step 13 to increase plasmid recovery efficiency. It is recommended to preheat the Buffer TB in a 65-70°C water bath before use. If the volume of elution buffer is less than 1 ml, it may affect recovery efficiency. DNA product should be stored at -20°C to avoid degradation. The pH value of elution buffer will have big influence in eluting. If using distilled water, pH should be controlled at 7.0-8.5, below 7.0 will affect elution efficiency.

An optional step

If you want to elevate the concentration of the plasmid, please finish the optional step.

14. Add 1.42 ml isopropanol and 0.42 ml 5M NaCl (prepared by the customer) to 1 ml plasmid elution buffer and mix completely. Incubate at room temperature for 5 min. Centrifuge for 10 min at 8,000 rpm (~8,228 × g), and discard the supernatant.
15. Add 0.5 ml 70% ethanol and Centrifuge for 10 min at 8,000 rpm (~8,228 × g), and then remove residual ethanol.
16. Repeat step 15.
17. Air-dry the washed plasmid at room temperature for 5-10 min, and add mall volume TB buffer to dissolve the plasmid.