EasyPrep EndoFree Maxi Plasmid Extraction kit V2

For purification of ultra-pure plasmid DNA

Contents

Introduction

TOOLS Endotoxin Free Maxi Plasmid kit V2 is based on alkaline lysis technology. DNA is adsorbed onto a silica membrane in the presence of a high salt concentration. This product is suitable for various routine applications, including restriction enzyme digestion, sequencing, library screening, ligation and transformation, *in vitro* translation, and the transfection of robust cells. With the use of an endotoxin removal buffer, the residual endotoxin can be as low as <0.1 EU/μg. The recommended bacterial culture volumes are 500–1500 μg of plasmid with 100 mL of bacterial culture for high-copy vectors and 50–300 μg of plasmid with 200 mL of bacterial culture for low-copy vectors.

Important Notes

1. Add the provided RNase A solution to Buffer PB1, mix, and store at 2°C–8°C.
2. Add ethanol (96%–100%) to Buffer PBW before use (check bottle label for volume).
3. Check Buffers BBL, PB2, and PB4 before use for salt precipitation. Redissolve any precipitate by warming to 37°C. Do not shake Buffer PB2 vigorously.
4. Prevent direct contact between Buffer PB2 and Buffer PB4. Close the bottle containing Buffer PB2 and Buffer PB4 immediately after use to avoid the acidification of both buffers (from contact with CO_2 in the air).
5. Draw out the plunger from the filter slowly to avoid loosening the membrane.
6. The amount of extracted plasmid is related to cell concentration and the plasmid copy. If working with low-copy vectors or large plasmids (>10 kb), it may be useful to increase the culture volume and increase Buffers PB1, PB2, and PB4 in proportion. Warm Buffer TB to 50°C–60°C before use. To increase extraction efficiency, ensure the that adsorption and elution times are prolonged appropriately.
7. After treatment with Buffer BBL, use Spin Column CP6 as soon as possible (within one day).

Kit Contents

Contents	DPT-BA20 (10 preps)
RNase A (10 mg/ml)	1.25 ml
Buffer BBL	30 ml
Buffer PB1	125 ml
Buffer PB2	125 ml
Buffer PB4	125 ml
Buffer PBW	50 ml
Buffer EBD	220 ml
Buffer TB	30 ml
Endotoxin removal buffer EBR	32 ml
Filtration Columns CS1	10
Spin Columns CP6	10
Collection Tubes（50 ml）	20

Storage

The TOOLS Endotoxin Free Maxi Plasmid kit V2 can be stored in a dry place at room temperature (15°C–25°C) for up to 12 months with no detriment to performance and quality. RNaseA (10 mg/mL) can be stored for 1 year at room temperature (15°C–25°C). After RNaseA is added, Buffer PB1 is stable for 6 months at 2°C–8°C.

Protocol

1. Column equilibration: place Spin Column CP6 into the 50-mL collection tube (supplied in the kit) and add 2.5 mL of Buffer BBL to Spin Column CP6. Centrifuge for 2 min at 8,000 rpm (≈8,228 × g). Discard the flow-through and finally place Spin Column CP6 into the same collection tube.
2. Harvest 100 mL of bacterial culture cells through centrifugation at 8,000 rpm (≈8,228 × g) for 3 min at room temperature (15°C–25°C) and then remove all traces of supernatant by inverting the open centrifuge tube until the medium is completely drained.
3. Resuspend pelleted bacterial cells in 10 mL of Buffer PB1.

Note: Ensure that RNase A has been added to Buffer PB1. No cell clumps should be visible after resuspension of the pellet.

4. Add 10 mL of Buffer PB2 and mix thoroughly by inverting the tube six to eight times and incubating at room temperature for 5 min.

Note: Mix by inverting the tube. Do not vortex because this will result in the shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to continue for more than 5 min.

5. Add 10 mL of Buffer PB4 and mix immediately and thoroughly by vigorously inverting six to eight times until the whole solution becomes cloudy. Incubate at room temperature for 10 min. A white fluffy material will form. Note: To avoid localized precipitation, mix the lysate thoroughly and immediately after the addition of Buffer PB4. Mix by inverting the tube. Do not vortex.
6. Centrifuge for 10 min at 8,000 rpm (≈8,228 × g). The white material will be precipitated to the bottom of the centrifuge tube and form a white pellet.

Note: Centrifugation time can be appropriately prolonged to precipitate the white material. If more than 100 mL of bacterial culture is used, prolong the centrifugal time to 20–30 min.

7. Transfer the supernatant into Filtration Column CS1. Gently insert the plunger into Filtration Column CS1 and filter the cell lysate into a new 50-mL tube (not supplied in the kit).

Note: If the white material fails to precipitate to the bottom in step 6, then the lysate was not mixed thoroughly in step 5. In this case, avoid transferring large clumps into Filtration Column CS1 to avoid clogging the filtration membrane. The presence of small fragments does not affect the applicability of Filtration Column CS1.

8. Add 3 mL of Buffer EBR (orange) into the tube and invert the tube until the color turns light yellow. Optional: For lower residual endotoxin, incubate the solution on ice for 20 min and then place the tube in a water bath (42°C) for 5 min. Centrifuge for 3 min at 5,000 rpm and transfer the supernatant into a new 50-mL tube.

9. Add 0.3-volume isopropanol (for low-copy vectors: 0.2-volume) to the cleared lysate, seal the received tube, mix completely, transfer the solution completely to Spin Column CP6, and finally centrifuge for 2 min at 8,000 rpm (≈8,228 × g).

Note: If the volume of the isopropanol–lysate mixture is larger than the capacity of the column, the mixture can be loaded into the column twice.

10. Add 10 mL of Buffer EBD 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 (for low-copy vectors, execute step 10 twice).
11. Add 10 mL of Buffer PBW to the column and centrifuge at 8,000 rpm (≈8,228 × g) for 2 min. Discard the flow-through and place Spin Column CP6 back into the same collection tube.
12. Repeat step 11.
13. Add 3 mL of 96%-100% ethanol to Spin Column CP6 (put CP6 in a collection tube). Centrifuge for 2 min at 8,000 rpm (≈8,228 × g).
14. Discard the flow-through and centrifuge at 8,000 rpm (≈8,228 × g) for an additional 5 min to remove residual ethanol.

Note: Residual ethanol will influence the subsequent enzymatic reaction and sequence. Air-dry the membrane by airing the column, with the cap open, for several minutes.

15. To elute DNA, place the column in the clean 50-mL collection tube (supplied in the kit), add 1–2 mL of Buffer TB into the center of the membrane, incubate 5 min at room temperature, and finally centrifuge at 8,000 rpm (≈8,228 × g) for 5 min.

Note: Repeat step 12 to increase plasmid callback efficiency. If the volume of the eluted buffer is less than 1 mL, recovery efficiency may be affected. The pH of the eluted buffer partially affects elution. Buffer TB or distilled water (pH: 7.0–8.5) is recommended for plasmid-DNA elution. For long-term DNA storage, elution in Buffer TB and storage at −20°C are recommended because if stored in water, DNA is subject to acid hydrolysis.