

EasyPure® EndoFree Pro Plasmid MaxiPrep Kit

Please read the manual carefully before use.

Cat. No. EM123

Version No. Version 3.0

Storage: at 15°C-30°C for two years.

Description

EasyPure® EndoFree Pro Plasmid MaxiPrep Kit uses a modified alkaline lysis method to isolate high quality plasmid DNA from *E. coli* cultured in ≤500 ml of LB medium. The solution contains indicators that can indicate whether the lysis and neutralization are complete through the change of color, so as to ensure the quality of plasmid extraction and visualize the operation. Plasmid purification process include special endotoxin removal solution. A newly developed endotoxin removal solution capable of reducing endotoxin levels to as low as 0.001 EU/μg. The purified DNA is suitable for a variety of molecular biology applications including restriction enzyme digestion, ligation, transformation, DNA sequencing, and transfection.

Features

- Visualized operation: solution LB (blue) indicates whether the lysis and neutralization are complete through the change of color.
- Fast: the whole procedure can be performed in one hour.
- High purity: high-purity, endotoxin-free (<0.1EU/μg) transfection-grade plasmid DNA can be prepared.
- High yield (up to 4 mg nucleic acid load in the purification column).

Self-prepared

Isopropanol (analytically pure), absolute ethanol (analytically pure), high-speed centrifuge, thermostatic water bath, 50 ml centrifuge tubes.

Kit Contents (10 rxns are counted by using 100 ml-200 ml LB media for experiments)

Component	EM123-01 (10 rxns)
Resuspension Buffer (RB)	120 ml
Lysis Buffer (LB, Blue)	120 ml
Neutralization Buffer 1 (NB1)	120 ml
Activation Buffer (AB)	55 ml
Endotoxin Removal Buffer (ER)	36 ml
ToxinOut Buffer 1 (TB1)	110ml
Wash Buffer (WB)	25 ml
Elution Buffer (EB)	25 ml
RNase A (10 mg/ml)	1.2 ml
Maxi-Plasmid Spin Column with Collection Tube	10 each
Push Filter	10 each
50 ml Collection Tube	10 each

Procedures (Using bacterial cultures grown in 100–200 ml of LB medium as an example)

Prior to use, add RNaseA to RB, store at 2-8°C; add 100 ml of absolute ethanol to WB.

1. Centrifuge 100–200 ml of overnight bacterial culture (grown for 14–16 hours, OD600 ≈ 3.0) at 8,000 × g for 4 minutes. Carefully discard the supernatant. (For low-copy plasmids, the volume of the bacterial liquid can be appropriately increased to obtain better extraction effect)
2. Column activation: add 5 ml of column activation solution AB to the center of Maxi-Plasmid Spin Column with Collection Tube, centrifuge at 8,000 × g for 1 minute, discard the effluent and set aside.(Activated spin columns should be used as soon as possible within 1 hour)



3. Add 10 ml of colorless solution RB (containing RNase A). Mix thoroughly by vortexing. And there should be no small bacterial masses.
4. Add 10 ml of blue solution LB, gently flip up and down and mix 5-8 times (violent shaking will cause genomic DNA contamination), so that the bacteria are fully lysed. The color changes from semi-translucent to translucent blue, indicating complete lysis (should not exceed 5 minutes).
5. Add 10 ml of NB1 to the step 4 lysate and mix gently 6-8 times (supernatant color changes from blue to colorless, indicating complete neutralization) until a compact clump is formed. Stay still for 2 minutes at room temperature.
6. Centrifuge at $10,000 \times g$ for 15 minutes (more bacteria can appropriately extend the centrifugation time), carefully avoid precipitation. Pour the supernatant into the Push Filter and push into a new 50 ml centrifuge tube (self-provided). (Slowly pull up the filter rod to prevent the filter from loosening and affecting the filter effect.)
7. Add 3 ml of the orange solution ER to the supernatant and mix upside down 5 times until an orange suspension is formed.
8. Add 0.3 volumes of isopropanol based on the volume after ER has been added and mix upside down. Transfer the liquid to the spin column in several times. Centrifuge at $8,000 \times g$ each time for 1 minute. Discard the effluent. (It is recommended that the volume of a single column should not exceed 17 ml, and the height of the liquid should not exceed the " $\triangle MAX \triangle$ " line. Multiple centrifuges can be used to avoid liquid spillage.)
9. Add 10 ml of solution TB1 and centrifuge at $8,000 \times g$ for 1 minute. Discard the effluent.
10. Add 10 ml of solution WB and centrifuge at $8,000 \times g$ for 1 minute. Discard the effluent.
11. Centrifuge at $8,000 \times g$ for 3 minutes to thoroughly remove residual WB. Place the spin column in a new 50 ml Collection Tube. Leave the column open for 5 minutes at room temperature to allow the ethanol to evaporate clean.
12. Add 1-2 ml of EB or deionized water ($7.0 < pH < 8.5$) to the center of the centrifuge column and stay still for 5 minutes at room temperature. (EB or deionized water is better used after preheating in a water bath at $60-70^{\circ}C$).
13. Centrifuge the column at $8,000 \times g$ for 2 minutes to elute DNA. (To increase plasmid DNA recovery, repeat this step by adding the eluate back to the center of the spin column)
14. Isolated plasmid DNA can be stored at $-20^{\circ}C$.

Optional (to further concentrate DNA)

1. Transfer eluent to a microcentrifuge tube, add 1/10 volume of NB1 and 7/10 volume of isopropanol (room temperature). Mix thoroughly and incubate at room temperature for 5 minutes.
2. Centrifuge at $12,000 \times g$ for 10 minutes and discard the supernatant (If the precipitate is not tightly adhered to the wall, the centrifugation time can be extended).
3. Add 1 ml of 70% ethanol (room temperature), centrifuge at $12,000 \times g$ for 10 minutes and discard the supernatant. Then brief centrifugation and aspirate the residual liquid.
4. Air-dry the pellet for 5-10 minutes. Add appropriate volume of EB to dissolve the pellet.

Notes

- All centrifugation steps are carried out at room temperature.
- After adding LB or NB1, mix gently. Vigorous mix may result in genome contamination.
- Add the whole volume of RNase A (supplied with this kit) into RB solution, mix thoroughly and store at $2-8^{\circ}C$.
- Prior to use, check whether the LB is cloudy or not, if it is cloudy, heat it in $37^{\circ}C$ water bath to completely dissolve it. Close the cap immediately after each use to avoid pH change.
- To obtain plasmid of high quality, please strictly control cell culture input volume. Please strictly follow the table below for RB, LB, NB1 and ER volume usage. If the OD600 of the bacterial solution exceeds 3.0 or the volume of bacteria is too large, it will lead to incomplete lysis and insufficient endotoxin removal performance, influencing the plasmid DNA yield and purity. The dosage of RB, LB, NB1 and ER can be increased in the same proportion.
- RB, LB, NB1, ER, RNase A (10 mg/ml) can be purchased separately, the catalog number is EM123-00, in case of insufficient reagent. See the appendix for details.



Bacterial biomass			Recommended reagent volume			
Bacterial liquid (OD ₆₀₀ ≈3.0)	OD ₆₀₀ *V (Volume, ml)	Wet weight of bacterial pellet	RB	LB	NB1	ER
< 100 ml	300	< 0.4g	5 ml	5 ml	5 ml	1.5 ml
100 ml-200 ml	600	0.4 g ≤ Bacterial pellet < 0.8 g	10 ml	10 ml	10 ml	3 ml
200 ml-300 ml	900	0.8 g ≤ Bacterial pellet < 1.2 g	15 ml	15 ml	15 ml	4.5 ml
300 ml-400 ml	1200	1.2 g ≤ Bacterial pellet < 1.6 g	20 ml	20 ml	20 ml	6 ml
400 ml-500 ml	1500	1.6 g ≤ Bacterial pellet < 2.0 g	25 ml	25 ml	25 ml	7.5 ml

- The elution volume should not be less than 1ml, and too small the elution volume will affect the elution efficiency.
- After extraction, plasmid DNA is recommended to be detected by agarose gel electrophoresis (presence or absence of RNA, genomic DNA residue, proportion of plasmid supercoiled conformation). Residues of RNA or genomic DNA will result in inflated plasmid concentration values, affecting quantitative accuracy and downstream applications.
- When extracting low copy plasmids and large plasmids larger than 10 kb, it is recommended to increase the amount of bacterial input to obtain better plasmid DNA yield.

Appendix

Component	EM123-00
Resuspension Buffer (RB)	120 ml
Lysis Buffer (LB, Blue)	120 ml
Neutralization Buffer 1 (NB1)	120 ml
Endotoxin Removal Buffer (ER)	36 ml
RNase A (10 mg/ml)	1.2 ml

For research use only, not for clinical diagnosis.

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