EasyPure® EndoFree Plasmid MaxiPrep Kit

Please read the datasheet carefully prior to use.

Cat. No. EM122

Version No. Version 3.0

Storage at 15℃-30℃ for one year.

Description

EasyPure® EndoFree Plasmid MaxiPrep Kit uses alkaline lysis method to extract plasmid DNA from no more than 500 ml of LB *E. coli* culture. Unique silica membrane adsorption technique to efficiently bind plasmid DNA. The solution contains indicators that can indicate whether the lysis and neutralization are complete through the change of color, so as to visualize the procedure. Endotoxin is removed on the column, which is fast and simple to use. 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, so as to ensure the quality of plasmid extraction.
- Fast: the whole procedure can be performed within one hour.
- Easy to use: on-column rapid endotoxin removal.
- High yield (up to 4 mg nucleic acid load in the purification column).

Self-prepared

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

Kit Contents (The calculation for 10 rxns is based on using 100-200 ml of bacterial culture in LB Media per reaction.)

Component	EM122-01 (10 rxns)
Resuspension Buffer (RB)	120 ml
Lysis Buffer (LB, Blue)	120 ml
Neutralization Buffer 1 (NB1)	120 ml
Activation Buffer (AB)	55 ml
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 (Take 100~200 ml of bacterial solution cultured with LB Media as an example)

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

1. Transfer 100 ml-200 ml overnight bacterial culture (OD_{600}≈3.0) into a centrifuge tube, centrifuge it at 8,000×g for 4 minutes, and discard the supernatant. (For low-copy plasmids, the volume of the bacterial culture 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 flow through 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 (vigorous 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 solution 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. Incubate at room temperature for 5 minutes.
6. Centrifuge at 10,000 × g for 15 minutes (extend the centrifugation time appropriately if there is a mount of bacteria). Carefully avoid precipitation, pour the supernatant into the Push Filter and push into a new 50 ml centrifuge tube (self-prepared). (Slowly pull up the filter rod to prevent the filter from loosening and affecting the filter effect.)
7. Add 0.3 times the volume of isopropanol (Analytical Reagent) to the filtrate and mix upside down. Transfer the liquid to the spin column in several times. Centrifuge at 8,000 × g each time for 1 minute. Discard the flow through. (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 "△MAX△" line. Multiple centrifuges can be used to avoid liquid spillage.)
8. Add 5 ml of solution WB. Centrifuge at 8,000 × g for 1 minute. Discard the flow through.
9. Repeat step 8 once.
10. Centrifuge at 8,000 × 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.
11. Dropping 1-2 ml of EB or deionized water (7.0<pH<8.5) to the center of the centrifuge column and stand at room temperature for 5 minutes (EB or deionized water is better used after preheating in a water bath at 60-70 °C).
12. Centrifuge the column at 8,000 × g for 2 minutes to elute DNA (To increase the recovery rate of plasmid DNA, add the eluate back to the center of the spin column and repeat this step).
13. Eluted plasmid DNA can be stored at -20 °C.

Optional (to further concentrate DNA)

1. Transfer eluent to a centrifuge tube, add 1/10 volume of NB1 and 7/10 volume of isopropanol (room temperature). Mix well and incubate at room temperature for 5 minutes.
2. Centrifuge at 12,000×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×g for 10 minutes and discard the supernatant. Then briefly centrifuge 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, the operation should be gentle. Vigorous mixing will lead to genome contamination.
- Add the whole volume of RNase A (supplied in this kit) into RB solution, mix thoroughly and store at 2-8°C.
- Prior to use, check whether the LB is turbid or not, if it is turbid, it should be heated in 37°C water bath to make it clear. 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 and NB1 volume usage. If the OD_{600} of the bacterial solution exceeds 4.0 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 and NB1 can be increased in the same proportion. RB, LB, NB1, RNase A (10 mg/ml) can be purchased separately, the catalog number is EM122-00, 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
＜100 ml	300	＜0.4g	5 ml	5 ml	5 ml
100 ml-200 ml	600	0.4 g ≤Bacterial pellet＜0.8 g	10 ml	10 ml	10 ml
200 ml-300 ml	900	0.8 g ≤ Bacterial pellet＜1.2 g	15 ml	15 ml	15 ml
300 ml-400 ml	1200	1.2 g ≤ Bacterial pellet＜1.6 g	20 ml	20 ml	20 ml
400 ml-500 ml	1500	1.6 g ≤ Bacterial pellet ＜2.0 g	25 ml	25 ml	25 ml

- The elution volume should not be less than 1ml, too small elution volume will affect the elution efficiency.
- It is recommended to detect the quality of the extracted plasmid DNA by agarose gel electrophoresis (with or without RNA or genomic DNA residue, and the proportion of supercoiled conformation of the plasmid). Residues of RNA or genomic DNA will lead to artificially high plasmid concentration, affecting quantitative accuracy and downstream applications.
- When extracting low-copy plasmids and large plasmids larger than 10kb, it is recommended to increase the volume of bacterial solution to obtain better plasmid DNA yield.

Appendix

Component	EM122-00
Resuspension Buffer (RB)	120 ml
Lysis Buffer (LB, Blue)	120 ml
Neutralization Buffer 1 (NB1)	120 ml
RNase A (10 mg/ml)	1.2 ml

For research use only, not for clinical diagnosis.

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