EasyPure® EndoFree Plasmid MaxiPrep Kit

Please read the manual carefully before use.

Cat. No. EM123

Version No. Version 1.1

Storage:at room temperature (15-25 ℃) under dry conditions for one year.

Description

EasyPure® EndoFree Plasmid MaxiPrep Kit uses a modified alkaline lysis method to isolate high quality plasmid DNA from≤500 ml(LB) of bacterial culture. 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 by the endotoxin removal solution. The purified DNA is suitable for a variety of molecular biology applications including restriction enzyme digestion, ligation, transformation, DNA sequencing, and transfection. The kit can be used 10 times based on a single 100 ml to 200 ml bacterial input.

Features

- Operational visualization: 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

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)	33 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
Push Filter	10
50 ml Collection Tube	10

Procedures

Prior to use, add RNaseA to RB, store at 2-8°C; add 100 ml of 96-100% ethanol to WB.

LB Media	RB	LB	NB1	ER
≤ 100 ml	5 ml	5 ml	5 ml	1.5 ml
100 ml-200 ml	10 ml	10 ml	10 ml	3 ml
200 ml-300 ml	15 ml	15 ml	15 ml	4.5 ml
300 ml-400 ml	20 ml	20 ml	20 ml	6 ml
400 ml-500 ml	25 ml	25 ml	25 ml	7.5 ml

1.Incubate the bacterial solution overnight for 14-16 hours. Centrifuge bacterial liquid (OD600≈3.0) at 10,000 × g for 2 minutes and 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 min, discard the effluent and set aside.
3.Add colorless solution RB (containing RNase A). Mix thoroughly by vortexing. And there should be no small bacterial masses.
4.Add 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 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. Ice bath for 2 min.
6.Centrifuge 10,000 × 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).
7.Add orange solution ER to the supernatant and mix upside down 5 times until an orange suspension is formed.
8.Add 0.3 times the volume of isopropanol to the obtained aqueous phase 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 effluent. (When the inclination angle of the centrifuge rotor is large, the volume of a single column should not exceed 10 ml, and multiple centrifuges can be used to avoid liquid spillage)
9.Add 5 ml of solution WB. Centrifuge 8,000 × g for 1 minute. Discard the effluent.
10.Repeat step 9 once.
11.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.
12.Add 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).
13.Centrifuge the column at 8,000 × 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°C.

Optional (to futher concentrate DNA)

1. Transfer eluent to a microcentrifuge tube, add 1/10 volume of NaAC (3 M, pH 5.2) and 7/10 volume of isopropanol (at room temperature). Mix thoroughly and incubate at room temperature for 5 minutes.
2. Centrifuge at 12,000×g for 10 minutes and discard the supernatant.
3. Add 1 ml of 70% ethanol (room temperature), centrifuge at 12,000×g for 10 minutes and discard the supernatant.
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 NB, don't mix by vortexing. 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°C.
- Prior to use, check whether the LB is cloudy or not, if it is cloudy, heat it in 37°C water bath to completely dissolve it. Close the cap immediately after each use to avoid pH change.

- Make sure to use the right cell culture volume. Too much cell culture can result in incomplete lysis, which will affect plasmid DNA yield and purity.
- 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.
- To obtain plasmid DNA with lower endotoxin levels: place the orange suspension from step 7 in an ice bath for 10 minutes. Then transfer to a 60°C water bath for 5 minutes. Centrifuge at 8,000×g for 4 minutes at room temperature.Use the pipette tips against the wall of the tube to discard the upper orange oily phase.

For research use only, not for clinical diagnosis.

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