AllPure EndoLow Plasmid MaxiPrep Kit

Please read the datasheet carefully prior to use.

Cat. No: ABTGNA306-10

Version No. Version 1.0

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

Description

AllPure EndoLow 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 special TB Buffer can effectively remove endotoxin. 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. The kit can be used 10 times based on a single 100 ml to 200 ml bacterial input.cterial input.

Features

- Visual Procedure: 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).

Self-prepared

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

Kit Contents

Component	ABTGNA306-10(10 rxns)
Resuspension Buffer (RB)	120 ml
Lysis Buffer (LB, Blue)	120 ml
Neutralization Buffer 1 (NB1)	120 ml
Activation Buffer (AB)	55 ml
ToxinOut Buffer (TB)	60 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 RNase A to RB, store at 2-8℃; add 100 ml of absolute ethanol to WB.

LB Media	RB	LB	NB1
≤ 100 ml	5 ml	5 ml	5 ml
100 ml-200 ml	10 ml	10 ml	10 ml
200 ml-300 ml	15 ml	15 ml	15 ml
300 ml-400 ml	20 ml	20 ml	20 ml
400 ml-500 ml	25 ml	25 ml	25 ml

1. Take the overnight cultured bacterial solution, centrifuge it at 8,000 × g for 4 minutes, and discard the supernatant (remove as completely as possible).
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 flow through and set aside.
3. According to the table above, add colorless solution RB (containing RNase A). Mix thoroughly by vortexing. And there should be no small bacterial masses.
4. According to the table above, add 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. According to the table above, 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. 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).
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. (When the inclination angle of the centrifuge rotor is large, the volume of a single column should not exceed 10 ml, and multiple centrifuging can be operated to avoid liquid spillage)
8. Add 5 ml of solution TB to the spin column. Incubate at room temperature for 10 minutes. Centrifuge at 8,000×g for 1 minute, and discard the flow through.
9. Add 5 ml of solution WB. Centrifuge at 8,000 × g for 1 minute. Discard the flow through.
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. 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 ℃).
13. 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).
14. Eluted plasmid DNA can be stored at -20 ℃.

Optional (to further 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 (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 NB1, the operation should be gentle. Vigorous mixing will lead to genome contamination.
- Add the whole volume of RNaseA (supplied in this kit) into RB solution, mix thoroughly and store at 2-8℃.
- Prior to use, check whether the LB is turbid or not, if it is turbid, it should be heated in 37℃ water bath to make it clear. Close the cap immediately after each use to avoid pH change.
- The volume of bacterial should not exceed 500 ml. Please strictly follow the instructions for the dosage of RB, LB, and NB1. If the volume of bacterial is too much, it will lead to insufficient lysis, which will affect the yield and purity of plasmid DNA.
- 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.