EasyPure® HiPure Plasmid MiniPrep Kit

Cat. No. EM111
Version No. Version 2.0
Storage: at 15℃-30℃ in a dry place for two years.

Description

EasyPure® HiPure Plasmid MiniPrep Kit uses a modified alkaline lysis method to lyse *E. coli* cells and utilizes silica membrane spin columns to specifically adsorb DNA. It is suitable for high-efficiency plasmid DNA extraction from *E. coli* cultured in ≤20 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. Endotoxin is removed on the column, which is simple to use. The purified plasmid DNA is suitable for a variety of molecular biology applications, including restriction enzyme digestion, ligation, transformation, DNA sequencing, and transfection.

Features

• Visualized operation: solutions LB (blue) and NB (yellow) indicate whether the lysis and neutralization are complete through the change of color, ensuring high-quality plasmid extraction.
• Fast: the whole procedure can be performed in one hour.
• Simple: endotoxin is removed on column.
• High yield: DNA yield up to 40 μg.
• The extracted plasmid DNA is of high purity and endotoxin-free.

Kit Contents

Component	EM111-01 (50 rxns)
Resuspension Buffer (RB)	15 ml
Lysis Buffer (LB, Blue)	15 ml
Neutralization Buffer (NB, Yellow)	20 ml
ToxinOut Buffer (TB)	15 ml
Wash Buffer (WB)	10 ml
Elution Buffer (EB)	5 ml
RNase A (10 mg/ml)	150 μl
Mini-Plasmid Spin Columns with Collection Tubes	50 each

Procedures

Prior to use, add RNaseA to RB, store at 2-8℃; add 40 ml of 100% ethanol to WB.

1. Add overnight bacterial culture to a centrifuge tube. Centrifuge at 10,000×g for 1 minute and discard the supernatant (aspirate as completely as possible). If the bacterial culture volume is too large, perform multiple centrifugation steps to collect the pellets.

LB Media	RB	LB	NB
≤5 ml	250 μl	250 μl	350 μl
5~10 ml	500 μl	500 μl	700 μl
10~15 ml	750 μl	750 μl	1050 μl
15~20 ml	1000 μl	1000 μl	1400 μl

2. Add appropriate volume of RB (premixed with RNase A) to the cell pellet. Mix thoroughly by vortexing. And there should be no small bacterial masses.
3. Add appropriate volume of LB (Blue), gently mix by inverting the tube 4-6 times, so that the bacteria are fully lysed. The color changes from semi-translucent to translucent blue, indicating complete lysis (should not exceed 5 minutes).

4. Add appropriate volume of NB (Yellow), mix gently 5-6 times. (The color of the lysate will turn into yellow when the neutralization is complete and a yellowish precipitate will form). Incubate the lysate at room temperature for 2 minutes.
5. Centrifuge at 12,000×g for 5 minutes, gently transfer the supernatant to a spin column. Centrifuge at 12,000×g for 1 minute and discard the flow-through (for supernatant more than 800 µl, repeat the process).
6. Add 250 µl TB, incubate at room temperature for 10 minutes. Centrifuge at 12,000×g for 1 minute and discard the flow-through.
7. Add 650 µl of WB (check to make sure that ethanol has been added) to the column, Centrifuge at 12,000×g for 1 minute. Discard the flow through.
8. Centrifuge the column at 12,000×g for 1-2 minutes to remove residual WB completely.
9. Place the spin column in a clean centrifuge tube, add 30-50 µl of Elution Buffer or deionized water to the center of the column matrix (for higher yield, prewarm EB or ddH_2O at 60-70°C).
10. Centrifuge the column at 10,000×g for 1 minute to elute DNA. Isolated plasmid DNA can be stored at -20°C.

Notes

- All centrifugation steps are carried out at room temperature.
- After adding LB or NB, mix the mixture gently. Vigorous mix may result in genomic DNA contamination.
- Add all 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 at 37°C water bath to completely dissolve it. Tight the cap immediately after use to avoid pH change.
- Up to 40 µg DNA can be otained with the kit. If plasmid DNA yield is low, use more bacterial culture.
- Please follow the manual strictly regarding the volumes of RB, LB, and NB. Excessive culture volume may result in incomplete lysis, affecting both the yield and purity of plasmid DNA.
- 5 ml of bacterial culture is considered as 1 rxn.

For research use only, not for clinical diagnosis.

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