EasyPure® Plasmid MiniPrep Kit

Please read the datasheet carefully prior to use.

Cat. No. EM101

Version No. Version 2.0

Storage: at room temperature (15-25℃) in a dry place for one year

Description

EasyPure® Plasmid MiniPrep Kit uses a modified alkaline lysis method followed by adsorption of DNA onto silica membrane to isolate high-quality plasmid DNA from ≤20 ml cultures of E.coli in LB medium. DNA yield up to 40 μg. 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. The purified plasmid DNA is suitable for a variety of molecular biology applications, including restriction enzyme digestion, ligation, transformation and DNA sequencing.

Kit Contents

Component	EM101-02 (200 rxns)
Resuspension Buffer (RB)	60 ml
Lysis Buffer (LB, Blue)	60 ml
Neutralization Buffer (NB, Yellow)	80 ml
Wash Buffer (WB)	2×20 ml
Elution Buffer (EB)	10 ml
RNase A (10 mg/ml)	600 μl
Mini-Plasmid Spin Columns with Collection Tubes	2×100

Procedures

Before use, add the RNase A to RB and store at 2-8oC; add different volumes of 100% ethanol to WB.

- **50 rxns (40 ml)**
- **200 rxns (2×80 ml)**

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

1. Centrifuge overnight cultured bacterial at 10,000×g for 1 minute. Discard the supernatant as much as possible. If the amount of bacterial liquid is too large, it can be collected by centrifugation multiple times.
2. Add colorless solution RB (containing RNase A) according to the table above. Mix thoroughly by vortexing. And there should be no small bacterial masses.
3. Add blue solution LB according to the table above, gently flip up and down and mix 4-6 times, so that the bacteria are fully lysed and translucent blue solution is formed. The color changes from semi-translucent to translucent blue, indicating complete lysis (should not exceed 5 minutes).
4. Add yellow solution NB according to the table above and mix gently 5-6 times (supernatant color changes from blue to colorless, indicating complete neutralization) until a compact yellow clump is formed. Keep it still for 2 minutes at room temperature.
5. Centrifuge at 12,000×g for 5 minutes. Transfer the supernatant into a spin column. Centrifuge at 12,000×g for 1 minute. Discard the flow through. If the supernatant volume is greater than 800 μl, it can be divided into multiple centrifugations.

6. Add 650 µl of WB. Centrifuge at 12,000×g for 1 minute. Discard the flow through.
7. Centrifuge the empty column at 12,000×g for 1-2 minutes to remove residual WB completely.
8. Place the spin column in a clean microcentrifuge tube, add 30-50 µl of EB or deionized water (pH >7.0) directly to the center of the column (for higher yield, preheat EB or deionized water to 60-70°C). Incubate the column at room temperature for 1 minute.
9. Centrifuge the column at 10,000×g for 1 minute to elute DNA. The isolated plasmid DNA can be stored at -20°C.

Notes

- All centrifugation steps are carried out at room temperature.
- After adding LB or NB, the operation must be gentle. 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.
- Maximum DNA yield by this kit is 40 µg. If plasmid DNA yield is low, increase the volume of bacterial culture .
- Use the amount of RB, LB and NB as suggested in the manual. Too much cell culture can result in incomplete lysis, which will affect plasmid DNA yield and the purity.
- 5 ml LB Media is considered as 1 rxn.

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