EasyPure® Plasmid MiniPrep Kit

Cat. No. EM101
Version No. Version 2.0
Storage: at room temperature (15-25°C) in a dry place for one year

Description

EasyPure® Plasmid MiniPrep Kit uses a modified alkaline lysis method to isolate high-quality plasmid DNA from ≤20 ml (LB) of bacterial culture. Unique formulated lysis buffer and neutralization buffer permit complete bacterial cell lysis and neutralization. The purified plasmid DNA is suitable for a variety of molecular biology applications, including restriction enzyme digestion, ligation, transformation and DNA sequencing.

• Simple and fast: the whole procedure can be performed in 20 minutes.
• High yield: DNA yield up to 40 µg.

Kit Contents

Component	EM101-01 (50 rxns)	EM101-02 (200 rxns)
Resuspension Buffer (RB)	15 ml	60 ml
Lysis Buffer (LB)	15 ml	60 ml
Neutralization Buffer (NB)	20 ml	80 ml
Wash Buffer (WB)	10 ml	2×20 ml
Elution Buffer (EB)	5 ml	10 ml
RNase A (10 mg/ml)	150 µl	600 µl
Mini-Plasmid Spin Column with Collection Tubes	50 each	2×100 each

Procedures

1. Add overnight cultured bacterial suspension to a microcentrifuge tube.

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. Centrifuge at 10,000×g for 1 minute. Discard the supernatant.
3. Add appropriate volume of RB (premixed with RNase A) to the cell pellet and resuspend it completely by pipetting.
4. Add appropriate volume of LB (Blue) , mix immediately and thoroughly by inverting the tube 4-6 times.
   (After this step, the lysate should change from opaque to bright blue. Proceed the following steps within 5 minutes after this step.)
5. Add appropriate volume of NB (Yellow), mix thoroughly by inverting the tube 4-6 times. The lysate will turn yellow when the neutralization is complete and a yellowish precipitate will form. Incubate the lysate at room temperature for 2 minutes.
6. Centrifuge at 12,000×g for 5 minutes. Transfer the supernatant into a spin column.
7. Centrifuge at 12,000×g for 1 minute. Discard the flow through.
8. 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.
9. Centrifuge the empty column at 12,000×g for 1-2 minutes to remove residual WB completely.

10. Place the spin column in a clean microcentrifuge tube, add 30-100 µl of EB or sterile, distilled water (pH >7.0) directly to the center of the column matrix (for higher yield, preheat EB or water to 65°C). Incubate the column at room temperature for 1 minute. Centrifuge the column at 10,000×g for 1 minute to elute DNA. The isolated plasmid DNA is ready to use or can be stored at -20°C.

Notes

- All centrifugation steps are carried out at room temperature.
- Add the entire RNase A from its tube to one bottle of RB solution, mix well 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. Tight the cap immediately after 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.

For research use only, not for clinical diagnosis.

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