EasyPure® HiPure Plasmid MiniPrep Kit

Cat. No. EM 111
Version No. Version 2.0
Storage: at room temperature (15-25°C) in a dry place for one year.

Description

EasyPure® HiPure Plasmid MiniPrep Kit provides an efficient way to isolate high yield (up to 40 μg) and 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.Endotoxin is removed by a simple incubation on column with a novel buffer. The purified plasmid DNA is suitable for a variety of molecular biology applications, including restriction enzyme digestion, ligation, transformation, DNA sequencing, and transfection.

- Fast: the whole procedure can be performed in 20 minutes.
- Simple: endotoxin is removed on column.
- High yield: DNA yield up to 40 μg.

Kit Contents

Component	EM111-01 (50 rxns)
Resuspension Buffer (RB)	15 ml
Lysis Buffer (LB)	15 ml
Neutralization Buffer (NB)	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°C; add 40 ml of 96-100% ethanol to WB.

1. Add overnight bacterial culture to a microcentrifuge tube. Centrifuge at 10,000×g for 1 minute and discard the supernatant

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 and resuspend it completely by pipetting.
3. Add appropriate volume of LB (Blue), immediately mix by inverting the tube 4-6 times (the color of the lysate should change from opaque to bright blue).
4. Add appropriate volume of NB (Yellow), mix by inverting the tube 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 once).
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 microcentrifuge tube, add 30-50 µl of Elution Buffer or sterile, distilled water directly 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 is ready to use or 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.
- 5 ml LB Media is considered as 1 rxn.

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