MCLAB
Molecular Cloning Laboratories

User Manual

Version 2.1

Product name: Midi Plus and Maxi Plus Ultrapure Plasmid Extraction System

Cat #: PPMD-100, PPMD-200, PPMX-100, PPMX-200

Description:

Ultrapure Plasmid Extraction System allows for the isolation of ultrapure plasmid DNA from a large volume of sample culture. Plasmid DNA purified from our proprietary anion-exchange resin is suitable for the use in PCR reaction, transfection, automated sequencing, and enzymatic modifications.

Application:

Plasmid or cosmid DNA purified with Ultrapure Plasmid Extraction System is ideal for the use in following applications:

- Transfection
- Transformation
- Ligation and cloning
- Manual or automated sequencing, including radioactive and fluorescent sequencing
- In vitro transcription

Protocol:

1. Culture plasmid-containing bacterial cells in 25-50 ml (high-copy-number plasmids) or 100-200 ml (low-copy number plasmids) of LB medium. Grow 12-16 hours with vigorous shaking at 37°C.
2. Harvest the bacterial cells by centrifugation at 6,000 x g for 15 minutes.
3. Equilibrate columns by applying 3 ml of 98-100% ethanol. Allow the column to empty by gravity flow and discard the filtrate.
4. Apply 5 ml of VPN buffer to the column and allow it to flow through by gravity flow and discard the filtrate.
5. Resuspend the cell pellet in 4 ml of VP1 buffer.
6. Add 4 ml of VP2 buffer, mix gently by inverting the lysate and stand for 5 minutes.
7. Add 4 ml of ice-cold VP3 buffer, mix gently by inverting.
8. Centrifuge at 20,000 x g for 15 minutes at 4°C.
9. Apply the supernatant with plasmid DNA to the column and allow it to flow through by gravity flow and discard the filtrate.
10. Wash the column once with 15 ml of VPN buffer by gravity flow and discard the filtrate.
11. Apply 5 ml of VPE buffer to elute DNA by gravity flow.
12. Precipitate DNA by adding 3.5 ml (0.7 volumes) of room temperature isopropanol to elute. Mix and centrifuge at 15,000 x g for 30 minutes at 4°C. Carefully remove the supernatant.
13. Wash the DNA pellet with 5 ml of room temperature 70% ethanol and centrifuge at 15,000 x g for 10 minutes. Carefully remove the supernatant.
14. Air-dry the DNA pellet for 10 minutes and dissolve the DNA in 100 µl or selected volume of TE or ddH_2O.
15. (Optional Step) Some insoluble material may remain in the final product. To eliminate the insoluble material, load the dissolved DNA sample into a column (sitting in a 1.5 ml tube) and spin at full speed in a micro centrifuge for 20 seconds. Collect the eluted DNA sample in the 1.5 ml tube.
16. Store DNA at -20°C