

Quick Start Guide

GenElute™ Endotoxin-Free Plasmid Midiprep Kit

PLED35

Reagents to Prepare

- Spin RNase Solution briefly and add 500 µL of RNase A Solution to the Resuspension Solution.
- Add 100 mL of 95-100% ethanol to Wash Solution Concentrate. After each use, tightly cap the diluted Wash Solution to prevent ethanol evaporation.

Protocol

All spins at 3,000-5,000 x g.

Harvest Bacteria

1. Pellet cells from 5-40 mL overnight culture by spinning for 10 min. Discard supernatant.

Resuspend & Lyse Bacteria

2. Resuspend cells in 1.2 mL Resuspension Solution. Pipet up and down to mix.
3. Add 1.2 mL Lysis Solution. Gently invert 6-8 times to mix until solution becomes clear and viscous. Do not vortex or allow lysis to exceed 5 min.

Prepare Cleared Lysate

4. Add 0.8 mL Neutralization Solution to the lysed cells and gently invert 6-8 times. Do not vortex.
5. Pellet cell debris by spinning for 15 min at 15,000 x g at 2-8 °C. Transfer cleared lysate into 15 mL conical tube. Repeat spin if floating cell debris present.

Remove Endotoxin

6. Add 300 µL Endotoxin Removal Solution (pre-chilled) to lysate and mix by inverting for 1 min. Chill on ice for ≥5 min and mix 1-2 times during incubation. Warm in a 37 °C water bath for 5 min. Spin for 5 min. Carefully transfer the clear upper phase (plasmid DNA) into a fresh 15 mL conical tube and discard the lower blue phase. Repeat once.
7. Add 0.8 mL DNA Binding Solution to the endotoxin-free lysate and mix thoroughly by inverting or vortexing.

Prepare Column

8. Place column into a collection tube and add 3 mL Column Preparation Solution to each column and spin for 1-2 min. Discard flowthrough.

Bind DNA to Column

9. Transfer lysate to column and spin for 1-2 min. Discard flowthrough.

Wash to Remove Contaminants

10. (EndA+ strains only): Add 2 mL Optional Wash Solution to column. Spin 2 min. Discard flowthrough.
11. Add 3 mL of diluted Wash Solution to column and spin for 1-2 min. Discard flowthrough.

Elute Purified DNA

12. Transfer column to fresh collection tube. Add 1 mL Endotoxin Free Water to the column and spin for 3-5 min.

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