

Quick Start Guide

GenElute™ Endotoxin-Free Plasmid Maxiprep Kit

PLEX15

Reagents to Prepare

- Spin RNase Solution briefly and add 500 μ L of RNase A Solution to the Resuspension Solution.
- Add 200 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 \times g unless otherwise noted.

Harvest Bacteria

1. Pellet cells from 25-200 mL overnight culture by spinning for 10 min. Discard supernatant.

Resuspend and Lye Bacteria

2. Resuspend cells in 4.8 mL Resuspension Solution. Pipet up and down to mix and transfer to centrifuge tube.
3. Add 4.8 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 3.2 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 \times g at 2-8 °C. Transfer cleared lysate into 15 mL conical tube. Repeat spin if floating cell debris present.

Remove Endotoxin

6. Add 1.2 mL Endotoxin Removal Solution (pre-chilled) to lysate and mix by inverting for 1 min. Chill on ice for \geq 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 50 mL conical tube and discard the lower blue phase. Repeat once.
7. Add 3.2 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 12 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 8 mL optional Wash Solution to column. Spin 2 min. Discard flowthrough.
11. Add 15 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 3-5 mL Endotoxin Free Water to the column and spin for 3-5 min.

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