

## Quick Start Guide

# GenElute™ HP Endotoxin-Free Plasmid Megaprep Kit

## NA0600

### Reagents to Prepare

- Add 1.25 mL of RNase A Solution to the Resuspension Solution. Store at 2-8 °C.
- Add 300 mL of 95-100% ethanol to Wash Solution 2. After each use, tightly cap the diluted Wash Solution to prevent ethanol evaporation.

### Protocol

Vacuum format.

#### Harvest Bacteria

1. Pellet cells from 200-1000 mL overnight culture by spinning for 10 min at 5,000 x g. Discard supernatant.

#### Prepare Column

2. Place Column onto vacuum manifold.
3. Add 40 mL of Column Preparation Solution to column, apply vacuum and allow liquid to pass through.

#### Resuspend and Lyse Bacteria

4. Resuspend cells in 40 mL Resuspension Solution. Pipet to mix.
5. Add 40 mL Lysis Solution. Gently invert 6-8 times to mix. Do not vortex. Allow to clear for 3-5 min.

#### Prepare Cleared Lysate

6. Add 40 mL Neutralization Solution (chilled prior to use) to the lysed cells and gently invert 6-8 times to mix.
7. Attach the Lysate Filter to a 45 mm neck bottle ( $\geq 250$  mL).
8. Empty entire bag of Lysate Clearing Agent into lysate and gently invert 6-8 times to mix. Incubate 5 min at room temperature.
9. Add lysate to assembled Lysate Filter and apply vacuum to clear cell debris.
10. Add 50 mL Binding Solution to the bottle containing the cleared lysate and swirl to mix.

#### Bind DNA to Column

11. Add cleared lysate to prepared column, apply vacuum and allow all lysate to pass through.

### Wash to Remove Contaminants

12. Add 40 mL of Wash Solution 1 to column and allow it to pass through.
13. Add 60 mL Wash Solution 2 to column and allow it to pass through.
14. Leave vacuum on for 20-30 min to dry the column.

### Elute Purified DNA

15. Attach a VacCap to a 50 mL collection tube and attach the column to the VacCap.
16. For maximum plasmid yield, add 20 mL of Endotoxin-Free Water to column and apply vacuum for 2-3 min.
17. For maximum plasmid concentration, add 5 mL of Endotoxin-Free Water and apply vacuum for 2 mins. Add another 5 mL of Endotoxin-Free Water and apply vacuum for another 5 min.

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