# **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**

- 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 (≥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.
- 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.

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#### **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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