

Quick Start Guide

GenElute™ HP Select Plasmid Gigaprep Kit

NA0800

Reagents to Prepare

- Add 3.0 mL of RNase A Solution to the Resuspension Solution. Store at 2-8 °C.
- Add 800 mL of 95-100% ethanol to the larger bottle (200 mL size) of Wash Solution 2; should additional quantities be needed, 300 mL of 95-100% ethanol can be added to the smaller bottle (75 mL size) of 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 2000 mL overnight culture by spinning for 10 min at 5,000 x g. Discard supernatant.

Prepare Column

2. Place Column onto a 45 mm neck bottle (1000 mL).
3. Add 100 mL of Column Preparation Solution to column, apply vacuum and allow it to pass through.

Resuspend and Lyse Bacteria

4. Resuspend cells in 100 mL Resuspension Solution. Pipet or vortex to mix.
5. Add 100 mL Lysis Solution. Gently invert 10-12 times to mix. Do not vortex. Allow to clear for 3-5 min.

Prepare Cleared Lysate

6. Add 100 mL Neutralization Solution (chilled prior to use) to the lysed cells and gently shake 10-12 times to mix.
7. Empty entire bag of Lysate Clearing Agent into lysate and gently shake several times to mix. Incubate 5 min at room temperature.
8. Attach the Lysate Filter to a 45 mm neck bottle (≥500 mL).
9. Add lysate to assembled Lysate Filter and apply vacuum to clear cell debris.
10. Add 100 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 EndoCleaning Solution to column and allow it to pass through.
13. Add 150 mL Wash Solution 1 to the column and allow it to pass through.
14. Add 200 mL Wash Solution 2 to column and allow it to pass through.
15. Leave vacuum on for 25-35 min to dry the column.

Elute Purified DNA

16. Attach Column to another sterile Endo-Free 45 mm neck bottle (150-250 mL).
17. Add 30 or 50 mL Endotoxin-Free Water to column, wait 1-2 min and apply vacuum for 2-3 min.

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