

GENELUTE HP ENDOTOXIN-FREE PLASMID MAXIPREP KIT

- ❑ **Preparation:** See Technical Bulletin for Details
 - Add RNase A to Resuspension Solution
 - Add Ethanol to Wash Solution 2
 - Chill Neutralization Solution

1 Harvest & Lyse Bacteria

- ❑ Pellet **150 ml** of an overnight culture at 5,000 x g, 10 minutes. Discard supernatant.
- ❑ Resuspend cells in **12 ml** of Resuspension Solution. Pipet up and down, or vortex to mix.
- ❑ Add **12 ml** of Lysis Solution and gently invert 6-8 times to mix. Do not shake or vortex. Allow to clear, 3-5 minutes.

2 Filter Lysate

- ❑ Prepare lysate filter by placing a VacCap onto a collection tube and attaching an Endotoxin-Free Maxiprep Filter to the VacCap.
- ❑ Neutralize lysate by adding **12 ml** of chilled Neutralization Solution and gently invert 6-8 times to mix.
- ❑ Immediately pour the lysate into the filter assembly and incubate for 5 minutes.
- ❑ Filter lysate by attaching vacuum source to the VacCap and applying vacuum.

3 Bind Plasmid DNA

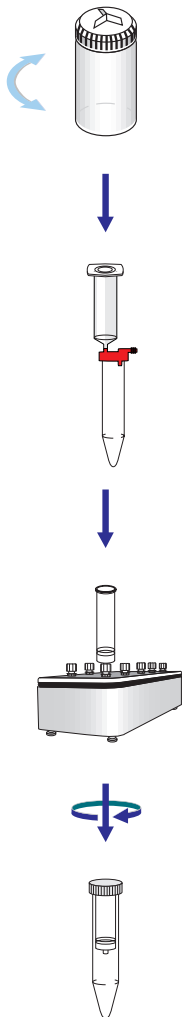
- ❑ Add **9 ml** of Binding Solution to the filtered lysate and gently invert 6-8 times.
- ❑ Place a Maxiprep Binding Column onto a vacuum manifold. Add **12 ml** of Column Preparation Solution to the binding column and vacuum through.
- ❑ Transfer lysate to the prepared column with the vacuum on. **Do not allow the lysate to pass below the surface of the binding material until all the lysate has been added to the column.**

4 Wash Column

- ❑ Add **12 ml** of Wash Solution 1 and vacuum through.
- ❑ Add **12 ml** of Wash Solution 2 and vacuum through.
- ❑ Leave the vacuum on for at least 10 minutes to dry the column. If more than six columns are on the manifold, dry for at least 20 minutes.

5 Elute Plasmid DNA

- ❑ Transfer the Binding Column to a collection tube.
- ❑ Add **3 ml** of Endotoxin-Free Water to the Binding Column.
- ❑ **For maximum yield of Plasmid DNA:** centrifuge the column/collection tube unit in a swinging bucket rotor at 3,000 x g for 5 minutes.
- ❑ **For maximum concentration of Plasmid DNA:** centrifuge the column/collection tube unit in a swinging bucket rotor at 1,000 x g for 5 minutes.



See Technical Bulletin for detailed procedure

