

## Experienced User Protocol

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

### 1 Harvest Bacteria

- ❑ Pellet **150 ml** of an overnight culture at 5000 x g, 10 minutes. Discard supernatant.

### 2 Prepare Column

- ❑ Place a Maxiprep Binding Column onto the Sigma VM20 vacuum manifold. Add **12 ml** of Column Preparation Solution to the binding column and vacuum through.

### 3 Resuspend & Lyse Bacteria

- ❑ Resuspend cells in **12 ml** of Resuspension/RNase 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.

### 4 Prepare Cleared Lysate

- ❑ Neutralize lysate by adding **12 ml** of chilled Neutralization Solution and gently invert 6–8 times to mix.
- ❑ Prepare lysate filter by placing a VacCap onto a collection tube and attaching an Endotoxin-Free Maxiprep Filter to the VacCap.
- ❑ 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.
- ❑ Add **9 ml** of Binding Solution to the filtered lysate and gently invert 6–8 times.

### 5 Bind Plasmid DNA to Column

- ❑ 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.**

### 6 Wash to Remove Contaminants

- ❑ 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.

### 7 Elute Purified 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 3000 x g for 5 minutes.
- ❑ **For maximum concentration of Plasmid DNA:** centrifuge the column/collection tube unit in a swinging bucket rotor at 1000 x g for 5 minutes.

