Midi and Maxi Plasmid Prep Using EconoSpin Silica Membrane Columns

Please note that Cat. No. 2030-050 and Cat. No. 2040-050 are silica membrane-based Midi and Maxi columns suitable for guanidine hydrochloride-based plasmid extraction. The binding capacity for these columns is 200 µg and 500 µg, respectively. For larger scale plasmid purification at the 3 mg to 10 mg level, please consider our Plasmid Plus Mega and Giga Spin Column 2030-PlusMega-050 and 2040-PlusGiga-050.

You will need a centrifuge with rotors that accept standard 15 mL and 50 mL culture tubes.

For high copy number plasmids, grow 50-300 mL of culture, and for low copy number plasmids, grow 100-500 mL of culture.

Proportionally enlarge the volume of each buffer (P1, P2, and P3) according to the mini prep protocol (250 µL P1: 250 µL P2: 350 µL P3 for 2-4 mL culture).

Follow the mini prep protocol up to the addition of P3.

1. Centrifuge at 4000- 10000 RPM for 10 minutes to precipitate protein and cell debris.
2. Load the supernatant onto the column at 4000 RPM for 1 minute
3. Wash twice with 10 ml Wash buffer at 4000 RPM for 1 minutes
4. Spin the column at top speed (8000-10000 RPM) for 20 minutes
5. Leave the column in room temperature for an additional 15 minutes to dry the column.
6. Insert the spin column into a new culture tube. Pre-warm the elution buffer to 50°C and load a minimum of 0.3 mL (for Midi column) or 3.0 mL (for Maxi column) onto the column, leaving it at room temperature for 5 minutes.
7. Centrifuge at top speed (8000-10000 RPM) for 2 minutes to collect the eluate.
8. Optional: If more concentrated DNA is needed, further ethanol precipitation of the eluted DNA is recommended.

Ethanol Precipitation Protocol

1. Measure the volume of the DNA sample.
2. Add 1/10 volume of sodium acetate, pH 5.2 (final concentration of 0.3 M).

3. Mix well.
4. Add 2 to 2.5 volumes of cold 100% ethanol (calculated after salt addition).
5. Mix well.
6. Place on ice or at -20°C for >20 minutes.
7. Spin at maximum speed (10000-15000 RPM) for 10-15 minutes.
8. Carefully decant the supernatant.
9. Add 1 mL of 70% ethanol. Do not disturb the pellet. Spin at top speed for 10 minutes. Carefully decant the supernatant.
10. Air-dry or briefly vacuum-dry the pellet.
11. Resuspend the pellet in the appropriate volume of TE or water.