Endo-Free Plasmid DNA Maxi Kit Vacuum Protocol

Before starting:

- Nuclease-free 50mL centrifuge tubes
- Set water bath to 42 °C
- Set water bath to 65 °C

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1. Transfer 50-200 mL overnight culture to an appropriate centrifuge bottle.
2. Centrifuge at 4000 g for 10 minutes at room temperature.
3. Discard the culture media.
4. Add 10 mL Solution I. Vortex to mix thoroughly.

   Note: Rnase A must be added to Solution I before use.
5. Add 10 mL Solution II. Invert and rotate the tube gently 8-10 times to obtain a cleared lysate. This requires a 2-3 minute incubation at room temperature with occasional mixing.
6. Add 5mL cold N3 Buffer. Gently invert until a flocculent white precipitate forms.

   Note: The solution must be mixed thoroughly. If the mixture still appears viscous, brownish, or conglobated, more mixing is required.
7. Centrifuge at 4000 g for 10 minutes at room temperature.
8. Prepare a lysate Clearance Filter Syringe by removing the plunger. Hold the barrel over a new 50 mL centrifuge tube. Transfer the lysate from Step 7 into the barrel.

   Note: Don’t transfer the precipitate into the battel. Keep the hand on the barrel.
9. Insert the plunger into the barrel to expel the cleared lysate into the 50 mL centrifuge tube.
10. Measure the volume of cleared lysate.
11. Add 0.1 volume ETR solution. Invert the tube gently 10 times. The lysate should appear turbid.

12. Incubate on -20 °C for 10 minutes.The lysate should become clear after incubation.
13. Incubate the lysate at 42 °C for 5 minutes. The lysate should appear turbid again.
14. Centrifuge at 4000 g for 5 minutes at 25 °C. The ETR Solution will form a blue layer at the bottom of the tube.

    Note: Set the deceleration rate of the centrifuge to 2.
15. Transfer the top aqueous phase to a new 50 mL tube.

    Note: No shaking, blowing, or pipetting into the centrifuge tube.
16. Add 0.5 volume absolute ethanol. Gently invert 6-7 times. Incubate at room temperature for 1-2 minutes.
17. Prepare the vacuum manifold. Turn off all columns.
18. Add 3 mL GPS buffer to each column. Let sit at room temperature for 5 minutes. Turn on the vacuum source to draw the buffer through the columns.
19. Transfer the cleared lysate from Step 16 to the columns.

    Note: Prepare HBC Buffer and DNA Wash Buffer.
20. Add 10 mL HBC Buffer.
21. Add 15 (13) mL DNA Wash Buffer.
22. Add 10 mL DNA Wash Buffer.
23. Turn off the vacuum. Transfer the column to a 50 mL Collection Tube.
24. Centrifuge at 4000 g for 10 minutes to dry the column matrix.
25. Transfer the column to a nuclease-free 50 mL centrifuge tube.
26. Dry at room temperature for 5 minutes.
27. Add 0.6 mL 65 °C water.
28. Centrifuge at 4000 g for 10 minutes.
29. Add 1 mL 65 °C water.
30. Centrifuge at 4000 g for 10 minutes.

31. Measure concentration at Nanodrop Spectrophotometer.

32. Store the aqueous phase in 1.5 mL EP tubes at -80 °C.