

Plasmid Miniprep

Adapted from BioBasic EZ-10 Spin Column Handbook

1. Add 1.5 - 5 mL of overnight culture to a 1.5 mL microcentrifuge tube and centrifuge at 12000 rpm for 2 minutes. Drain the liquid completely.
2. Add 100 μ L of Solution I to the pellet, mix well and keep for 1 minute.
3. Add 200 μ L of Solution II to the mixture, and mix gently by inverting the tube 4-6 times and keep at room temperature for 1 minute.
4. Add 350 μ L of Solution III and mix gently. Incubate at room temperature for 1 minute.
5. Centrifuge at 12000 rpm for 5 minutes.
6. Transfer the above supernatant to the EZ-10 column. Centrifuge at 10000 rpm for 2 minutes.
7. Discard the flow through in the tube. Add 750 μ L of Wash Solution to the column and centrifuge at 10000 rpm for 2 minutes.
8. Repeat wash procedure in step 7.
9. Discard the flow through in the collection tube. Centrifuge at 10000 rpm for an additional minute to remove any additional Wash Solution.
10. Transfer the column to a clean 1.5 mL microcentrifuge tube. Add 50 μ L of pre-warmed ddH₂O into the center part of the column and incubate at room temperature for 2 minutes. Centrifuge at 10000 rpm for 2 minutes.
11. Determine plasmid concentration and A260/A280 using the Montreal Biotech Inc. BioDrop
12. Store purified plasmid DNA at -20°C