

Adapted from BioBasic EZ-10 Spin Column Handbook

1. Excise the DNA fragment from the gel with a clean, sharp scalpel. Transfer the gel slice to a pre-weighed 1.5 mL microcentrifuge tube. Determine the gel slice weight.
2. Add 400 μ L of Binding Buffer II for each 100 mg of gel weight. Incubate at 50-60°C for 10 minutes and shake occasionally until agarose is completely dissolved.
3. Add the above mixture to the EZ-10 column and let stand for 2 minutes. Centrifuge at 10000 rpm for 2 minutes and discard the flow through in the tube
4. Add 750 μ L of Wash Solution and centrifuge at 10000 rpm for 1 minute. Discard the solution in the tube.
5. Repeat step 4. Centrifuge at 10000 rpm for an additional minute to remove any residual Wash Buffer.
6. Place the column in a clean 1.5 mL microcentrifuge tube. Add 30-50 μ L of pre-warmed ddH₂O to the center of the column and incubate at room temperature for 2 minutes. Centrifuge at 10000 rpm for 2 minutes to elute DNA.
7. Determine DNA concentration and A₂₆₀/A₂₈₀ using the Montreal Biotech Inc. BioDrop.
8. Store purified plasmid DNA at -20°C.