

DNA Purification from Enzymatic Reactions

Adapted from BioBasic EZ-10 Spin Column Handbook

1. Transfer reaction contents to a 1.5 mL microcentrifuge tube and add 3 volumes of Binding Buffer II. Mix by inverting the tube a few times.
2. Transfer the above mixture solution to the EZ-10 column and let stand at room temperature for 2 minutes. Centrifuge at 10000 rpm for 1 minute.
3. Remove the flow-through in the tube. Add 750 μ L of Wash Solution to the column and centrifuge at 10000 rpm for 1 minute.
4. Repeat washing procedure in step 3. Spin at 10000 rpm for an additional minute to remove any residual Wash Solution.
5. Transfer the column into a clean 1.5 mL microcentrifuge tube and add 30-50 μ L of pre-warmed ddH₂O. Incubate at room temperature for 2 minutes. Centrifuge at 10000 rpm for 2 minutes to elute the DNA.
6. Determine DNA concentration and A260/A280 using the Montreal Biotech Inc. BioDrop.
7. Store purified plasmid DNA at -20°C.