

Purpose: Extract plasmid DNA (smaller quantity)

QIAprep Spin Miniprep Kit Protocol

- a. Centrifuge 3 mL of bacterial overnight culture in two separate Eppendorf tubes (1.5 mL in each) at 8,000 rpm for 3 minutes at room temperature.
- b. Discard the supernatant and resuspend pelleted bacterial cells in one tube with 250 μ L Buffer P1 and transfer to the other and resuspend until one eppendorf tube contains the pelleted cells resuspended in 250 μ L Buffer P1.
- c. Add 250 μ L of Buffer P2 and invert 5 times.
- d. Add 350 μ L of Buffer N3 and immediately mix by inverting 5 times.
- e. Centrifuge for 10 minutes at 13,000 rpm.
- f. Micropipette 800 μ L of the clear supernatant into a spin column and centrifuge for 60 seconds and discard the excess liquid.
- g. Add 500 μ L of PB and centrifuge the spin columns for 60 seconds. Discard the flow through.
- h. Add 750 μ L of PE to the spin columns, centrifuge for 60 seconds, and discard the flow through.
- i. Centrifuge the spin columns again for 60 seconds to remove residual wash buffer and discard the flow through.
- j. Transfer the spin columns to a clean eppendorf tube and add 50 μ L of EB to the center of the spin column to elute the DNA.
- k. Allow the spin column to stand for one minute and then centrifuge for one minute.
- l. Record the concentrations for each sample.