QIAprep® Spin Miniprep Kit

The QIAprep Spin Miniprep Kit can be stored at room temperature (15–25°C) for up to 12 months. For more information, please refer to the most recent version of the QIAprep Miniprep Handbook, which can be found at: www.qiagen.com/handbooks.

Notes before starting

- Optional: Add LyseBlue reagent to Buffer P1 at a ratio of 1 to 1000.
- Add the provided RNase A solution to Buffer P1, mix and store at 2–8°C.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are carried out at 13,000 rpm (~17,900 x g) in a conventional
- table-top microcentrifuge.

1. Pellet 1–5 ml bacterial overnight culture by centrifugation at 8500 rpm for 3 min at room temperature (15–25°C).
2. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.
3. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times until the solution becomes clear.
    a. Do not allow the lysis reaction to proceed for more than 5 min. If using LyseBlue reagent, the solution will turn blue.
4. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times.
    a. If using LyseBlue reagent, the solution will turn colorless.
5. Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.
6. Apply 800 µl supernatant from step 5 to the QIAprep 2.0 spin column by pipetting.
    a. For centrifuge processing, follow the instructions marked with a triangle (▲).
    b. For vacuum manifold processing, follow the instructions marked with a circle (●).
    c. ▲ Centrifuge for 30–60 s and discard the flow-through
    d. ● Apply vacuum to the manifold to draw the solution through the QIAprep 2.0 spin column and switch off the vacuum source.
7. Recommended: Wash the QIAprep 2.0 spin column by adding 0.5 ml Buffer PB.
    a. ▲ Centrifuge for 30–60 s and discard the flow-through
    b. ● Apply vacuum to the manifold to draw the solution through the QIAprep 2.0 spin column and switch off the vacuum source.
    c. **Note:** This step is only required when using endA+ strains or other bacteria strains with high nuclease activity or carbohydrate content.
8. Wash the QIAprep 2.0 spin column by adding 0.75 ml Buffer PE.

a. ▲Centrifuge for 30–60 s and discard the flow-through
b. ● Apply vacuum to the manifold to draw the solution through the QIAprep 2.0 spin column and switch off the vacuum source.
c. Transfer the QIAprep 2.0 spin column to the collection tube.

9. Centrifuge for 1 min to remove residual wash buffer.
10. Place the QIAprep 2.0 column in a clean 1.5 ml microcentrifuge tube.
    a. To elute DNA, add 50 µl Buffer EB (10 mM TrisCl, pH 8.5) or water to the center of the QIAprep 2.0 spin column.
    b. Let stand for 1 min.
    c. Centrifuge for 1 min.
11. If the extracted DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA.
    a. Mix the solution by pipetting up and down before loading the gel.