Mini Prep

Project: iGEM2026
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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.

Protocol Steps:

1. Pellet 1–5 ml bacterial overnight culture by centrifugation at >8000 rpm (6800 x g) 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. 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. 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 the supernatant from step 5 to the QIAprep spin column by decanting or pipetting. S Centrifuge for 30–60 s and discard the flow-through, or z apply vacuum to the manifold to draw the solution through the QIAprep spin column and switch off the vacuum source.
7. Recommended: Wash the QIAprep spin column by adding 0.5 ml Buffer PB. S Centrifuge for 30–60 s and discard the flow-through, or z apply vacuum to the manifold to draw the solution through the QIAprep spin column and switch off the vacuum source. 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 spin column by adding 0.75 ml Buffer PE. S Centrifuge for 30–60 s and discard the flow-through, or z apply vacuum to the manifold to draw the solution through the QIAprep spin column and switch off the vacuum source. Transfer the QIAprep spin column to the collection tube.
9. Centrifuge for 1 min to remove residual wash buffer.
10. Place the QIAprep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.

https://www.qiagen.com/en-US/resources/download/kitHandbookAndProtocol/qiaprep-spin-miniprep-kit-en