QIAprep® Spin Miniprep Kit High-Yield Protocol

For purification of up to 30 µg plasmid DNA

This protocol is for use with the QIAprep Spin Miniprep Kit (cat. nos. 27104 and 27106), and is specially optimized for high-yield purification of high-copy plasmid DNA from bacterial cultures grown to a high cell density (e.g., culture grown in 5 ml 2x YT medium). For further details, including safety information, refer to the *QIAprep Miniprep Handbook*.

Note: Certain insert–plasmid constructs may reduce the copy number of the plasmid, resulting in lower yields after preparation. In addition, not all *E. coli* strains are suitable for high-yield plasmid purification. This protocol was verified using *E. coli* DH5α carrying pCMVβ plasmid.

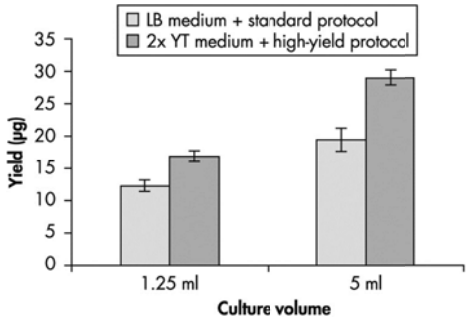

Figure 1. Higher plasmid DNA yields from cultures grown in 2x YT medium and isolated using the high-yield protocol. Plasmid DNA was isolated from *E. coli* DH5α (carrying plasmid pCMVβ) cultured in LB medium or 2x YT medium, using the QIAprep Spin Miniprep standard protocol and the high-yield protocol, respectively.

Notes before starting

- 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, unless stated otherwise, are carried out at 13,000 rpm (~17,900 x *g*) in a conventional table-top microcentrifuge.
- Symbols: ● centrifuge processing; ▲ vacuum processing.

Procedure

1. Inoculate 5 ml 2x YT medium with the bacterial strain and incubate the culture for 12–16 h at 37°C. Avoid overgrowth, which may lead to reduced plasmid yields.

2. Pellet 5 ml bacterial overnight culture by centrifugation at >8000 rpm (6800 x g) for 3 min at room temperature (15–25°C).
3. Completely resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube. Ensure that no clumps of cells remain.
4. Add 250 µl Buffer P2 and mix by inverting the tube 10–12 times until the solution turns completely blue. Incubate for 5 min. Do not allow lysis to proceed for more than 5 min.
5. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 10–12 times until the solution turns colorless.
6. Centrifuge for 10 min.
7. Apply the supernatant from step 6 to the QIAprep spin column by decanting or pipetting. ● Centrifuge for 30–60 s and discard the flow-through, or ▲ apply vacuum to the manifold to draw the solution through the QIAprep spin column and switch off the vacuum at source.
8. Wash the QIAprep spin column by adding 500 µl Buffer PB. ● Centrifuge as in step 7, or ▲ apply vacuum to draw the solution through the QIAprep spin column as in step 7.
9. Wash the QIAprep spin column by adding 750 µl Buffer PE. ● Centrifuge as in step 7, or ▲ apply vacuum to draw the solution through the QIAprep spin column as in step 7. Transfer the QIAprep spin column to the collection tube.
10. Centrifuge for 1 min to remove residual wash buffer.
11. Place the QIAprep spin column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 60 µl Buffer EB (10 mM Tris·Cl, pH 8.5) to the center of the QIAprep spin column, let it stand for 1 min, and centrifuge for 1 min.

Ordering Information

Product	Contents	Cat. no.
QIAprep Spin Miniprep Kit (50)	For 50 high-purity plasmid minipreps: 50 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)	27104
QIAprep Spin Miniprep Kit (250)	For 250 high-purity plasmid minipreps: 250 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)	27106

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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