QIAprep Spin Miniprep Kit Protocol

using a microcentrifuge

This protocol is designed for purification of up to 20 µg of high-copy plasmid DNA from 1–5-ml overnight cultures of *E. coli* in LB (Luria-Bertani) medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on page 31.

! **Please read Important Notes for QIAprep Procedures on pages 14–15 before starting.**

Procedure

1. **Resuspend pelleted bacterial cells in 250 µl of Buffer P1 and transfer to a microfuge tube.**

   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.

2. **Add 250 µl of Buffer P2 and gently invert the tube 4–6 times to mix.**

   Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

3. **Add 350 µl of Buffer N3 and invert the tube immediately but gently 4–6 times.**

   To avoid localized precipitation, mix the solution gently but thoroughly, immediately after addition of Buffer N3. The solution should become cloudy.

4. **Centrifuge for 10 min.**

   A compact white pellet will form.

   During centrifugation, place a QIAprep spin column in a 2-ml collection tube.

5. **Apply the supernatants from step 4 to the QIAprep column by decanting or pipetting.**

6. **Centrifuge 30–60 sec. Discard the flow-through.**

7. **(Optional): Wash QIAprep spin column by adding 0.5 ml of Buffer PB and centrifuging 30–60 sec. Discard the flow-through.**

   This step is necessary to remove trace nuclease activity when using *endA*$^+$ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α™ do not require this additional wash step.

8. **Wash QIAprep spin column by adding 0.75 ml of Buffer PE and centrifuging 30–60 sec.**

9. **Discard the flow-through, and centrifuge for an additional 1 min to remove residual wash buffer.**

   ! **IMPORTANT:** Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

10. **Place QIAprep column in a clean 1.5-ml microfuge tube. To elute DNA, add 50 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or H_2O to the center of each QIAprep column, let stand for 1 min, and centrifuge for 1 min.**

QIAprep Spin Miniprep Kit Protocol

using 5-ml collection tubes

The QIAprep Spin Miniprep procedure can be performed using 5-ml centrifuge tubes (e.g., Greiner, Cat. No. 115101 or 115261) as collection tubes to decrease handling. The standard protocol on pages 18–19 should be followed with the following modifications:

Step 4: Place QIAprep spin column in a 5-ml centrifuge tube instead of a 2-ml collection tube.

Step 6: Centrifuge at 3000 x *g* for 1 min using a suitable rotor (e.g., Beckman® GS-6KR centrifuge at ~4000 rpm). (The flow-through does not need to be discarded).

Steps 7 & 8: For washing steps, centrifugation should be performed at 3000 x *g* for 1 min. (The flow-through does not need to be discarded).

Step 9: Transfer QIAprep column to a microfuge tube. Microcentrifuge at maximum speed for 1 min. Continue with step 10 of the protocol.

QIAprep Spin Miniprep Kit Protocol

using a vacuum manifold

This protocol is designed for purification of up to 20 µg high-copy plasmid DNA from 1–5-ml overnight cultures of *E. coli* grown in LB (Luria-Bertani) medium, using QIAprep spin columns on QIAvac 6S or other vacuum manifolds with luer connectors. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on page 31.

! **Please read Important Notes for QIAprep Procedures on pages 14–15 before starting.**

Procedure

1. **Resuspend pelleted bacterial cells in 250 µl of Buffer P1 and transfer to a microfuge tube.**

   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuption of the pellet.

2. **Add 250 µl of Buffer P2 and invert the tube gently 4–6 times to mix.**

   Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

3. **Add 350 µl of Buffer N3 and invert the tube immediately but gently 4–6 times.**

   To avoid localized precipitation, immediately after addition of Buffer N3 mix the solution gently but thoroughly. The solution should become cloudy.

4. **Centrifuge for 10 min.**

   A compact white pellet will form.

   During centrifugation, prepare the vacuum manifold and QIAprep columns:

   QIAvac 6S manifold: (Note: The following procedure applies to the manifold with a hinged lid and spring lock. See Appendix A on page 32).

   - Open QIAvac 6S lid. Place QIAvac Luer Adapter(s), or blanks to seal unused slots, into the slots of the QIAvac top plate. Close the QIAvac 6S lid. Place the waste tray inside the QIAvac base, and place the top plate squarely over the base. Attach the QIAvac 6S to a vacuum source.
   - Insert each QIAprep spin column into a luer connector on the Luer Adapter(s) in the vacuum manifold. Seal unused luer connectors with plugs provided with the QIAvac Luer Adapter Set.

   Other vacuum manifolds: Follow the supplier's instructions. Insert each QIAprep column into a luer connector.

5. **Apply the supernatant from step 4 to the QIAprep column by decanting or pipetting.**

6. **Switch on vacuum source to draw the solution through the QIAprep column, and then switch off vacuum source.**
7. **(Optional): Wash QIAprep column by adding 0.5 ml of Buffer PB. Switch on vacuum source. After the solution has moved through the column, switch off vacuum source.**

   This step is necessary to remove trace nuclease activity when using *endA*$^+$ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α do not require this additional wash step.
8. **Wash QIAprep column by adding 0.75 ml of Buffer PE. Switch on vacuum source to draw the wash solution through the column, and then switch off vacuum source.**
9. **Transfer the QIAprep columns to a microfuge tube. Centrifuge for 1 min.**

   ! **IMPORTANT:** This extra spin is necessary to remove residual Buffer PE. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.
10. **Place QIAprep column in a clean 1.5-ml microfuge tube. To elute DNA, add 50 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or H_2O to the center of the QIAprep column, let stand for 1 min, and centrifuge for 1 min.**