January 2022

Quick-Start Protocol

QIAwave Plasmid Miniprep Kit

The QIAwave Plasmid Miniprep Kit (cat. no. 27206) can be stored at room temperature (15–25°C) for up to 12 months.

Further information

- *QIAwave Plasmid Miniprep Handbook*: **www.qiagen.com/HB-2991**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Preparation of Buffer PE/C: Transfer the entire volume of Buffer PE/C from the 15 ml bottle into a glass bottle larger than 275 ml, either by using a pipet or by pouring. Add 50 ml ultrapure water such as nuclease-free water (1000 ml, cat. no.129115; 5 liters, cat. no. 129117) and 220 ml ethanol (96–100%) to obtain a final volume of 275 ml. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, peel off the upper label from the piggyback label on the 15 ml plastic bottle and transfer it onto the glass bottle.
- Preparation of Buffer P1/C: Transfer the entire volume of Buffer P1/C from the 15 ml bottle into a glass bottle larger than 70 ml, either by using a pipet or by pouring. Add 60 ml ultrapure water such as nuclease-free water (1000 ml, cat. no.129115; 5 liters, cat. no. 129117) to obtain a final volume of 70 ml. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, peel off the upper label from the piggyback label on the 15 ml plastic bottle and transfer it onto the glass bottle.
- Preparation of Buffer EB/C: Transfer the entire volume of Buffer EB/C from the 15 ml bottle into a glass bottle larger than 55 ml, either by

using a pipet or by pouring. Add 50 ml ultrapure water such as nuclease-free water (1000 ml, cat. no.129115; 5 liters, cat. no. 129117) to obtain a final volume of 55 ml. Cap the glass bottle tightly and mix by inverting the bottle several times. To label the glass bottle, peel off the upper label from the piggyback label on the 15 ml plastic bottle and transfer it onto the glass bottle.

- **Optional**: Add LyseBlue® reagent to Buffer P1 at a ratio of 1 to 1000.
- Add 700 µl of the provided RNase A solution (conc.10 mg/ml) to the 70 ml reconstituted Buffer P1/C for a final concentration of 100 µg/ml. Mix and store at 2–8°C.
- All centrifugation steps are carried out at 13,000 rpm (approx. 17,900 *x g*) in a conventional table-top microcentrifuge.
- Preassemble QIAprep® 2.0 Spin Columns with Waste Tubes.

Procedure

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/C 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 (approx. 17,900 x *g*) in a table-top microcentrifuge.
6. Apply 800 µl supernatant from step 5 to the QIAprep 2.0 Spin Column placed into a 2 ml Waste Tube (supplied) by pipetting. For centrifuge processing, follow the instructions marked with a triangle (▲). For vacuum manifold processing, follow the instructions marked with a circle (●). ▲ Centrifuge for 30–60 s and discard the flow-

through, or ● 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. ▲ Centrifuge for 30–60 s and discard the flow-through, or ● apply vacuum to the manifold to draw the solution through the QIAprep 2.0 Spin Column and switch off the vacuum source.

   Note: This step is only required when using *end*A+ 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/C. ▲ Centrifuge for 30–60 s and discard the flow-through, or ● apply vacuum to the manifold to draw the solution through the QIAprep 2.0 Spin Column and switch off the vacuum source. 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 Spin Column in a clean 1.5 ml microcentrifuge tube (not supplied). To elute DNA, add 50 µl Buffer EB/C (10 mM TrisCl, pH 8.5) or water to the center of the QIAprep 2.0 Spin Column, let stand for 1 min, and centrifuge for 1 min.

Document Revision History

Date	Changes
01/2022	Initial release

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.