Quick-Start Protocol March 2016

CompactPrep® Plasmid Kits

CompactPrep Plasmid Kits (cat. nos. 12843 and 12863) can be stored at room temperature (15–25°C) for up to 2 years if not otherwise stated on label.

Further information

- *CompactPrep Plasmid Purification Handbook*: **www.qiagen.com/HB-1159**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Add RNase A solution to Buffer P1 to a final concentration of 100 µg/ml.
- **Optional**: Add LyseBlue® reagent to Buffer P1 at a ratio of 1:1000.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- Dissolve precipitates in Buffer P2 and Buffer BB by warming to 37°C.
- Close the bottle containing Buffer P2 immediately after use.
- All microcentrifugation steps are carried out at 10,000 x *g* (approximately 13,000 rpm) in a conventional tabletop microcentrifuge.
- Symbols: ■ 200 µg high-copy plasmid DNA using the CompactPrep Plasmid Midi Kit; ▲ 750 µg plasmid DNA using the CompactPrep Plasmid Maxi Kit.

Table 1. Maximum recommended Luria Bertani (LB) culture volumes

Plasmid	CompactPrep Plasmid Midi	CompactPrep Plasmid Maxi
High-copy	25 ml	100 ml
Low-copy	50 ml	250 ml

1. Harvest the bacterial cells by centrifugation at 6000 x *g* for 15 min at 4°C.
2. Completely resuspend the bacterial pellet in ■ 2 ml or ▲ 5 ml Buffer P1.

3. Add ■ 2 ml or ▲ 5 ml Buffer P2, mix thoroughly by vigorously inverting the sealed tube 4–6 times and incubate at room temperature (15–25°C) for 3 min. If LyseBlue reagent has been added, the cell suspension will turn blue.
4. Add ■ 2 ml or ▲ 5 ml Buffer S3 to the lysate, and mix immediately by vigorously inverting 4–6 times. Proceed directly to step 5. Do not incubate the lysate on ice. If LyseBlue reagent has been added, mix the solution until it is completely colorless.
5. Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Remove supernatant containing plasmid DNA promptly.
6. Centrifuge the supernatant again at ≥20,000 x *g* for 15 min at 4°C. Remove supernatant containing plasmid DNA promptly.
7. During incubation, prepare the vacuum manifold and CompactPrep Midi or Maxi columns.
8. Add ■ 2 ml or ▲ 5 ml Buffer BB to the lysate. Mix by inverting 4–6 times and transfer the adjusted lysate into a tube extender attached to the CompactPrep column.
9. Switch on vacuum source to draw the solution through the CompactPrep column and then switch off vacuum source.
10. Wash the DNA using a microcentrifuge (step 10a) or using a vacuum manifold (step 10b).

    10a. To wash the DNA using a microcentrifuge: Discard the tube extenders and place the CompactPrep column into a 2 ml collection tube. Wash by adding 0.7 ml Buffer PE and centrifuging for 30–60 s. Discard the flow-through, and centrifuge for an additional 1 min.

    10b. To wash the DNA using a vacuum manifold: Discard the tube extenders. Add 0.7 ml Buffer PE and switch on the vacuum manifold. To completely remove residual buffer, apply vacuum for an additional 10 min.
11. Place the CompactPrep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add ■ 100 µl or ▲ 200 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the CompactPrep column, let stand for 1 min then centrifuge for 1 min.

Scan QR code for handbook.