Quick-Start Protocol March 2016

QIAGEN® Plasmid *Plus* Midi Kit

The QIAGEN Plasmid *Plus* Midi Kit (cat. nos. 12943 and 12945) can be stored at room temperature (15–25°C) for up to 24 months if not otherwise stated on label.

Further information

- *QIAGEN Plasmid* Plus *Purification Handbook*: **www.qiagen.com/HB-0155**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Add RNase A solution to Buffer P1, mix and store at 2–8°C.
- **Optional**: Add LyseBlue® reagent to Buffer P1 at a ratio of 1:1000.
- Add ethanol (96–100%) to Buffer PE concentrate before use (see bottle label for volume).
- Harvest bacterial culture after 12–16 h incubation.
- Symbols: ● standard protocol; ▲ high-yield protocol.

Table 1. Maximum recommended LB culture volumes

Protocol	High-copy plasmid	Low-copy plasmid
Standard	20–25 ml	50 ml
High-yield	25–35 ml	Not recommended

1. Harvest bacterial culture by centrifuging at 6000 x *g* for 15 min at 4°C.
2. Completely resuspend pelleted bacteria in ● 2 ml or ▲ 4 ml Buffer P1.

3. Add ● 2 ml or ▲ 4 ml Buffer P2, gently mix by inverting until the lysate appears viscous and incubate at room temperature (15–25°C) for 3 min. If LyseBlue reagent has been added, the cell suspension will turn blue.
4. Place the QIAfilter Cartridge into a new and suitable tube, allowing space for the addition of Buffer BB.
5. Add ● 2 ml or ▲ 4 ml Buffer S3 to the lysate, and mix by inverting 4–6 times. If LyseBlue reagent has been added, mix the solution until it is completely colorless.
6. Transfer the lysate to the QIAfilter Cartridge and incubate at room temperature for 10 min.
7. During incubation, place QIAGEN Plasmid *Plus* spin columns into the QIAvac 24 Plus. Insert Tube Extenders into each column.
8. Gently insert the plunger into the QIAfilter Cartridge and filter the cell lysate into the tube.
9. Add 2 ml Buffer BB to the cleared lysate, and mix by inverting 4–6 times.
10. Transfer lysate to a QIAGEN Plasmid *Plus* spin column on the QIAvac 24 Plus.
11. Apply approximately –300 mbar vacuum until the liquid has been drawn through all columns.
12. To wash the DNA, add 0.7 ml Buffer ETR and apply vacuum until the liquid has been drawn through all columns.
13. To further wash the DNA, add 0.7 ml Buffer PE and apply vacuum until the liquid has been drawn through all columns.
14. To completely remove the residual wash buffer, centrifuge the column at 10,000 x *g* (9,700 rpm) for 1 min in a tabletop microcentrifuge.
15. Place the QIAGEN Plasmid *Plus* spin column into a clean 1.5 ml tube. To elute the DNA, add 200 µl Buffer EB or water to the center of the QIAGEN Plasmid *Plus* spin column, let it stand for ≥1 min and centrifuge for 1 min.

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