Quick-Start Protocol October 2015

QIAGEN® Plasmid *Plus* Maxi Kit

The QIAGEN Plasmid *Plus* Maxi Kit (cat. nos. 12963 and 12965) can be stored at room temperature (15–25°C) for up to 24 months.

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.
- Check Buffer P2 for SDS precipitation.
- 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; Δ high-yield protocol.

Table 1. Maximum recommended LB culture volumes

Protocol	High-copy plasmid	Low-copy plasmid
Standard	80–100 ml	Up to 200 ml
High-yield	100–130 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 • 5 ml or Δ 8 ml Buffer P1.
3. Add • 5 ml or Δ 8 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, mix the solution until it is homogeneously blue in color.

4. Add • 5 ml or Δ 8 ml Buffer S3 to the lysate. Mix immediately by inverting 4–6 times. If LyseBlue reagent has been added, mix the solution until it is completely colorless.
5. Transfer the lysate to the QIAfilter cartridge. Incubate at room temperature for 10 min.
6. During incubation, place QIAGEN Plasmid *Plus* spin columns into the QIAvac 24 Plus. Insert Tube Extenders into each column.
7. Gently insert the plunger into the QIAfilter Cartridge and filter the cell lysate into a new tube, allowing space for the addition of Buffer BB.
8. Add 5 ml Buffer BB to the cleared lysate, and mix by inverting 4–6 times.
9. Transfer the lysate to a QIAGEN Plasmid *Plus* spin column with a tube extender attached on the QIAvac 24 Plus.
10. Apply approximately –300 mbar vacuum until the liquid has been drawn through all columns. Switch off vacuum.
11. To wash the DNA, add 0.7 ml Buffer ETR and apply vacuum until the liquid has been drawn through all columns. Switch off vacuum.
12. Add 0.7 ml Buffer PE and apply vacuum until the liquid has been drawn through all columns. Switch off vacuum.
13. To completely remove residual wash buffer, place the QIAGEN Plasmid *Plus* Maxi spin column into a 2 ml collection tube provided and centrifuge the column at 10,000 *x g* (9,700 rpm) for 1 min in a tabletop microcentrifuge.
14. Place the QIAGEN Plasmid *Plus* spin column into a clean 2 ml tube. To elute the DNA, add 400 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.

Scan QR code for handbook.