Quick-Start Protocol March 2016

QIAGEN® Plasmid *Plus* Mega and Giga Kits

The QIAGEN Plasmid *Plus* Mega Kit (cat. no. 12981), and the QIAGEN Plasmid *Plus* Giga Kit (cat. no. 12991) 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 (see bottle label for volume).
- For QIAfilter Mega-Giga cartridges, use only appropriate bottles.
- Symbols: ● QIAGEN Plasmid *Plus* Mega Kit; ▲ QIAGEN Plasmid *Plus* Giga Kit using the QIAvac 24 Plus.

Table 1. Maximum recommended LB culture conditions

Kit	Culture volume	Incubation time
QIAGEN Plasmid *Plus* Mega	500 ml	12–16 h
QIAGEN Plasmid *Plus* Giga	2.5 l	12–16 h

1. Harvest bacterial culture by centrifuging at 6000 x *g* for 15 min at 4°C.

2. During centrifugation, screw the QIAfilter Cartridge onto a 45 mm-neck glass bottle and connect it to a vacuum source. Assemble the QIAGEN Plasmid *Plus* spin columns with the Tube Extenders and position them on the QIAvac 24 Plus assembled with the QIAvac Holder.
3. Completely resuspend pelleted bacteria in ● 25 ml or ▲ 100 ml Buffer P1.
4. Add ● 25 ml or ▲ 100 ml Buffer P2, gently mix by inverting and incubate at room temperature (15–25°C) for up to 5 min. If LyseBlue reagent has been added, the cell suspension will turn blue.
5. Add ● 25 ml or ▲ 100 ml Buffer S3 to the lysate, and mix by inverting 4–6 times. If LyseBlue reagent has been added, the lysate will turn colorless.
6. Transfer the lysate to the QIAfilter Cartridge and incubate at room temperature for 10 min.
7. Apply vacuum until liquid has been drawn through the QIAfilter Cartridge.
8. Add ● 25 ml or ▲ 100 ml Buffer BB, and mix by inverting 4–6 times.
9. Transfer lysate to a QIAGEN Plasmid *Plus* spin column on the QIAvac.
10. Apply approximately –300 mbar vacuum until the liquid has been drawn through all columns.
11. To wash the DNA, add 80 ml Buffer ETR and apply vacuum until the liquid has been drawn through all columns.
12. Add 50 ml Buffer PE and apply vacuum until the liquid has been drawn through all columns.
13. Transfer the QIAGEN Plasmid *Plus* spin column into a Collection Tube.
14. Centrifuge at 5000 x *g* for 10 min at room temperature to dry the membrane.
15. Place the QIAGEN Plasmid *Plus* spin column into a new Collection Tube. To elute the DNA, add ● 1 ml or ▲ 5 ml Buffer EB to the QIAGEN Plasmid *Plus* spin column, let it stand for at least 1 min and centrifuge at 5000 x *g* for 5 min at room temperature.

Scan QR code for handbook.