Quick-Start Protocol March 2016

QIAGEN® Plasmid Mega and Giga Kits

The QIAGEN Plasmid Mega Kit (cat. nos. 12181 and 12183) and the QIAGEN Plasmid Giga Kit (cat. no. 12191) can be stored at room temperature (15–25°C) for at least 2 years if not otherwise stated on label.

Further information

- QIAGEN Plasmid Purification Handbook: **www.qiagen.com/HB-1193**
- 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.
- Prechill Buffer P3 at 4°C. Check Buffer P2 for SDS precipitation.
- Isopropanol and 70% ethanol are required.
- Symbols: ● QIAGEN Plasmid Mega Kit; ▲ QIAGEN Plasmid Giga Kit.

Table 1. Maximum recommended LB culture volumes

Kit	High-copy plasmid	Low-copy plasmid
QIAGEN Plasmid Mega	500 ml	2.5 l
QIAGEN Plasmid Giga	2.5 l	5 l

1. Harvest bacterial overnight culture by centrifuging at 6000 x *g* for 15 min at 4°C.
2. Resuspend the bacterial pellet in ● 50 ml or ▲ 125 ml Buffer P1.

3. Add ● 50 ml or ▲ 125 ml Buffer P2, mix thoroughly by inverting 4–6 times and incubate at room temperature (15–25°C) for 5 min. If using LyseBlue reagent, the solution will turn blue.
4. Add ● 50 ml or ▲ 125 ml prechilled Buffer P3, mix thoroughly by vigorously inverting 4–6 times and incubate on ice for 30 min. If using LyseBlue reagent, mix the solution until it is colorless.
5. Centrifuge at ≥20,000 x *g* for 30 min at 4°C.
6. Optional: Re-centrifuge the supernatant at ≥20,000 x *g* for 15 min at 4°C if the solution is not clear after step 5.
7. Equilibrate a QIAGEN-tip ● 2500 or ▲ 10000 by applying ● 35 ml or ▲ 75 ml Buffer QBT.
8. Apply the supernatant from step 6 to the QIAGEN-tip.
9. Wash the QIAGEN-tip with a total of ● 200 ml or ▲ 600 ml Buffer QC.
10. Elute DNA with ● 35 ml or ▲ 100 ml Buffer QF into a clean vessel. For constructs larger than approximately 45 kb, prewarming the elution buffer to 65°C may help to increase the yield.
11. Precipitate DNA by adding ● 24.5 ml or ▲ 70 ml (0.7 volumes) room-temperature isopropanol and mix. Centrifuge at ≥15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant.
12. Wash the DNA pellet with ● 7 ml or ▲ 10 ml room-temperature 70% ethanol, centrifuge at ≥15,000 x *g* for 10 min. Carefully decant supernatant without disturbing the pellet.
13. Air-dry pellet for 10–20 min and redissolve DNA in a suitable volume of buffer. (e.g., TE buffer, pH 8.0, or 10 mM Tris·Cl, pH 8.5).

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. Trademarks: QIAGEN®, Sample to Insight®, LyseBlue® (QIAGEN Group). 1101273 03/2016 HB-0587-002 © 2016 QIAGEN, all rights reserved.