

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 for up to 6 months.
- **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.
- **Optional:** If using QIAfilter Mega-Giga Cartridges (cat. no. 19781) for lysate clearing, QIAfilter Mega-Giga Cartridges operate with any vacuum source that generates –200 to –600 mbar vacuum. Use only appropriate plastic or glass bottles that are designed for use under vacuum.

Table 1. Maximum recommended LB culture volumes

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

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. **Optional:** If using QIAfilter Mega-Giga Cartridges for lysate clearing, during the incubation of Buffer P2, screw the QIAfilter Cartridge onto a 45 mm neck glass bottle and connect it to a vacuum source.



5. 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.
6. **Optional:** If using QIAfilter Mega-Giga Cartridges for lysate clearing:
 - 6a. Pour the lysate into the QIAfilter Cartridge. Incubate at room temperature for 10 min. Apply vacuum until all the liquid has been pulled through. Leave the QIAfilter Cartridge attached.
 - 6b. Add 50 ml Buffer FWB2 (see Appendix B of the handbook) to the QIAfilter Cartridge and gently stir the precipitate using a sterile spatula. Apply vacuum until the liquid has been pulled through completely. Continue to step 9.
7. Centrifuge at $\geq 20,000 \times g$ for 30 min at 4°C.
8. **Optional:** Recentrifuge the supernatant at $\geq 20,000 \times g$ if the solution is not clear after step 5.
9. Equilibrate a QIAGEN-tip ● 2500 or ▲ 10000 by applying ● 35 ml or ▲ 75 ml Buffer QBT.
10. Apply the supernatant from step 6 (optional QIAfilter protocol) or step 8 (centrifugation protocol) to the QIAGEN-tip.
11. Wash the QIAGEN-tip with a total of ● 200 ml or ▲ 600 ml Buffer QC.
12. 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.
13. Precipitate DNA by adding ● 24.5 ml or ▲ 70 ml (0.7 volumes) room-temperature isopropanol and mix. Centrifuge at $\geq 15,000 \times g$ for 30 min at 4°C. Carefully decant the supernatant.
14. Wash the DNA pellet with ● 7 ml or ▲ 10 ml room-temperature 70% ethanol; centrifuge at $\geq 15,000 \times g$ for 10 min. Carefully decant supernatant without disturbing the pellet.
15. 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).

Document Revision History

Date	Changes
02/2021	Corrected the QIAGEN Plasmid Giga Kit catalog number. Added QIAfilter protocol.

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). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

1123554 02/2021 HB-0587-003 © 2021 QIAGEN, all rights reserved.