Quick-Start Protocol February 2025

EndoFree® Plasmid Mega and Giga Kit

The EndoFree Plasmid Mega Kit (cat. no. 12381) and the EndoFree Plasmid Giga Kit (cat. no. 12391) can be stored at room temperature (15–25°C) for up to 2 years if not otherwise stated on label.

Further information

- *EndoFree Plasmid Purification Handbook*: **www.qiagen.com/HB-1194**
- 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.
- Add 40 mL 96–100% ethanol to the endotoxin-free water supplied with the kit.
- Use endotoxin-free or pyrogen-free plasticware (step 9 onward).
- QIAfilter Mega-Giga cartridges: use only appropriate plastic or glass bottles that are
- designed for use under vacuum.
- Symbols: ■ EndoFree Plasmid Mega Kit; ▲ EndoFree Plasmid Giga Kit.

Procedure

1. Harvest overnight LB 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. During the incubation, 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 chilled Buffer P3, and mix thoroughly by vigorously inverting 4–6 times. If using LyseBlue reagent, mix the solution until it is completely colorless.

6. Pour the lysate into the QIAfilter Cartridge and incubate for 10 min. Apply vacuum until all liquid has been pulled through. Leave the QIAfilter Cartridge attached. Add 50 mL Buffer FWB2 to the QIAfilter Cartridge and gently stir the precipitate using a sterile spatula. Apply vacuum until the liquid has completely passed through.
7. Add ■ 18 mL or ▲ 40 mL Buffer ER (approx. 10% of the flitrated lysate volume) to the filtered lysate, mix by inverting the bottle approximately 10 times and incubate at room temperature for 5 min.
8. Equilibrate a ■ QIAGEN-tip 2500 or ▲ QIAGEN-tip 10000 by applying ■ 35 mL or ▲ 75 mL Buffer QBT, and allow the column to empty by gravity flow.
9. Apply the filtered lysate from step 7 to the QIAGEN-tip, and allow it to enter the tip.
10. Wash the QIAGEN-tip with ■ 200 mL or ▲ 600 mL Buffer QC.
11. Elute DNA with ■ 35 mL or ▲ 100 mL Buffer QN.
12. Precipitate DNA by adding ■ 24.5 mL or ▲ 70 mL (0.7 volumes) room-temperature isopropanol to the eluted DNA and mix. Centrifuge at ≥15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant.
13. Wash the DNA pellet with ■ 7 mL or ▲ 10 mL of endotoxin-free room-temperature 70% ethanol and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.
14. Air-dry the pellet for 10–20 min, and redissolve the DNA in a suitable volume of endotoxin-free Buffer TE.

Document Revision History

Date	Changes
02/2025	Edited according to new brand style guide. Change in step 7

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®,EndoFree®,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.
1136776 02/2025 HB-0589-003 © 2025 QIAGEN, all rights reserved.