Quick-Start Protocol March 2016

EndoFree® Plasmid Maxi Kit

The EndoFree Plasmid Maxi Kit (cat. no. 12362) 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.
- Isopropanol is required.
- 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).

Table 1. Maximum recommended LB culture volumes

Kit	High-copy plasmid	Low-copy plasmid
EndoFree Plasmid Maxi	100 ml	250 ml

1. Harvest overnight LB culture by centrifuging at 6000 x *g* for 15 min at 4°C.
2. Completely resuspend the bacterial pellet in 10 ml Buffer P1.
3. Add 10 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 cap onto the outlet nozzle of the QIAfilter Cartridge. Place the QIAfilter Cartridge in a convenient tube or in a QIArack (cat. no. 19015).
5. Add 10 ml chilled Buffer P3, mix thoroughly by inverting 4–6 times. If using LyseBlue reagent, mix the solution until it is completely colorless.
6. Pour the lysate into the barrel of the QIAfilter Cartridge. Incubate at room temperature for 10 min. Do not insert the plunger! Remove the cap from the QIAfilter Cartridge outlet nozzle. Gently insert the plunger into the QIAfilter Cartridge and filter the cell lysate into a 50 ml tube.
7. Add 2.5 ml Buffer ER to the filtered lysate, mix by inverting the tube approximately 10 times, and incubate on ice for 30 min.
8. Equilibrate a QIAGEN-tip 500 by applying 10 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 2 x 30 ml Buffer QC.
11. Elute DNA with 15 ml Buffer QN into a 30 ml endotoxin-free or pyrogen-free tube.
12. Precipitate DNA by adding 10.5 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 5 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 5–10 min and redissolve the DNA in a suitable volume of endotoxin-free Buffer TE.

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