Quick-Start Protocol March 2016

QIAfilter Plasmid Midi and Maxi Kits

The QIAfilter Plasmid Midi Kit (cat. nos. 12243 and 12245), the QIAfilter Plasmid Maxi Kit (cat. nos. 12262 and 12263) and the QIAfilter Midi Cartridges (cat. no. 19743) can be stored at room temperature (15–25°C) for at least 2 years if not otherwise stated on label.

Further information

- *QIAfilter Plasmid Purification Handbook*: **www.qiagen.com/HB-1169**
- 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 to 4°C. Check Buffer P2 for SDS precipitation.
- Isopropanol and 70% ethanol are required.
- Symbols: ● QIAfilter Plasmid Midi Kit; ▲ QIAfilter Plasmid Maxi Kit.

Table 1. Recommended LB culture volumes

Kit	High-copy plasmid	Low-copy plasmid
QIAfilter Plasmid Midi	25 ml	50–100 ml
QIAfilter Plasmid Maxi	100 ml	250 ml

1. Harvest bacterial culture after 12–16 h of growth by centrifuging at 6000 x *g* for 15 min at 4°C.
2. Completely resuspend pellet in ● 4 ml or ▲ 10 ml Buffer P1.

3. Add ● 4 ml or ▲ 10 ml Buffer P2, mix by inverting the sealed tube 4–6 times and incubate at room temperature (15–25°C) for up to 5 min. If using LyseBlue reagent, the solution will turn blue.
4. During the incubation, screw the cap onto the outlet nozzle of the QIAfilter Cartridge, and place it in a convenient tube or a QIArack (cat. no. 19015).
5. Add ● 4 ml or ▲ 10 ml prechilled Buffer P3 to the lysate, and mix immediately and 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 up to 10 min. Do not insert the plunger!
7. Equilibrate the QIAGEN-tip by applying ● 4 ml or ▲ 10 ml Buffer QBT, and allow the column to empty by gravity flow.
8. Remove the cap from the QIAfilter Cartridge outlet nozzle. Gently insert the plunger into the QIAfilter Cartridge, and filter the cell lysate into the equilibrated QIAGEN-tip. Allow the lysate to enter the resin by gravity flow.
9. Wash the QIAGEN-tip with ● 2 x 10 ml or ▲ 2 x 30 ml Buffer QC.
10. Elute DNA with ● 5 ml or ▲ 15 ml Buffer QF. For constructs ≥45 kb, prewarming the elution buffer to 65°C may help to increase the yield.
11. Precipitate DNA by adding ● 3.5 ml or ▲ 10.5 ml room-temperature isopropanol, mix and centrifuge at 15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant.
12. Wash the DNA pellet with ● 2 ml or ▲ 5 ml room-temperature 70% ethanol and centrifuge at 15,000 x *g* for 10 min. Carefully decant the supernatant.
13. Air-dry the pellet for 5–10 min and redissolve DNA in a suitable volume of a slightly alkaline buffer (e.g., TE buffer, pH 8.0, or 10 mM Tris·Cl, pH 8.5).

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