Quick-Start Protocol March 2016

QIAfilter Plasmid Mega and Giga Kits

The QIAfilter Plasmid Mega Kit (cat. no. 12281), the QIAfilter Plasmid Giga Kit (cat. no. 12291) and the QIAfilter Mega-Giga Cartridges (cat. no. 19781) 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.
- Add LyseBlue® reagent to Buffer P1 at a ratio of 1:1000.
- Check Buffer P2 for SDS precipitation.
- Prechill Buffer P3 to 4°C.
- Isopropanol and 70% ethanol are required.
- 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.
- Symbols: ● QIAfilter Plasmid Mega Kit; ▲ QIAfilter Plasmid Giga Kit.

Table 1. Maximum recommended LB culture volumes

Kit	High-copy plasmid	Low-copy plasmid
QIAfilter Plasmid Mega	500 ml	2.5 l
QIAfilter Plasmid Giga	2.5 l	Not recommended

1. Pellet 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 ● 50 ml or ▲ 125 ml Buffer P1.

3. Add ● 50 ml or ▲ 125 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 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 to the lysate and mix by inverting 4–6 times. If using LyseBlue reagent, the solution will turn colorless.
6. 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.
7. Add 50 ml Buffer FWB2 to the QIAfilter Cartridge and gently stir the precipitate using a sterile spatula. Apply vacuum until the liquid has been pulled through completely.
8. Equilibrate the QIAGEN-tip 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 resin.
10. Wash the QIAGEN-tip with ● 2 x 100 ml or ▲ 2 x 300 ml Buffer QC.
11. Elute DNA with ● 35 ml or ▲ 100 ml Buffer QF. For constructs ≥45 kb, prewarming the elution buffer to 65°C may help to increase the yield.
12. Precipitate DNA by adding ● 24.5 ml or ▲ 70 ml room-temperature isopropanol, mix and 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 room-temperature 70% ethanol and centrifuge at 15,000 x *g* for 10 min. Carefully decant the supernatant.
14. Air-dry the pellet for 10–20 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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