Quick-Start Protocol March 2016

QIAGEN® Plasmid Mini, Midi and Maxi Kits

The QIAGEN Plasmid Mini Kit (cat. nos. 12123 and 12125), the QIAGEN Plasmid Midi Kit (cat. nos. 12143 and 12145), the QIAGEN Plasmid Maxi Kit (cat. nos. 12162, 12163 and 12165) and the Plasmid Buffer Set (cat. no. 19046) can be stored at room temperature (15–25°C) for up to 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.
- **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 Mini Kit; ▲ QIAGEN Plasmid Midi Kit; and ■ QIAGEN Plasmid Maxi Kit.

Table 1. Recommended LB culture volumes

Kit	High-copy plasmid	Low-copy plasmid
QIAGEN Plasmid Mini	3 ml	Not recommended
QIAGEN Plasmid Midi	25 ml	100 ml
QIAGEN Plasmid Maxi	100 ml	500 ml

1. Harvest overnight bacterial culture by centrifuging at 6000 x *g* for 15 min at 4°C.
2. Resuspend the bacterial pellet in ● 0.3 ml, ▲ 4 ml or ■ 10 ml Buffer P1.

3. Add ● 0.3 ml, ▲ 4 ml or ■ 10 ml Buffer P2, mix thoroughly by vigorously 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. Add ● 0.3 ml, ▲ 4 ml or ■ 10 ml prechilled Buffer P3, mix thoroughly by vigorously inverting 4–6 times. Incubate on ice for ● 5 min, ▲ 15 min or ■ 20 min. If using LyseBlue reagent, mix the solution until it is colorless.
5. ●: Centrifuge at 14,000–18,000 x *g* for 10 min at 4°C. Re-centrifuge if the supernatant is not clear. ▲ and ■: Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Re-centrifuge the supernatant at ≥20,000 x *g* for 15 min at 4°C.
6. Equilibrate a QIAGEN-tip ● 20, ▲ 100 or ■ 500 by applying ● 1 ml, ▲ 4 ml or ■ 10 ml Buffer QBT, and allow column to empty by gravity flow.
7. Apply the supernatant from step 5 to the QIAGEN-tip and allow it to enter the resin by gravity flow.
8. Wash the QIAGEN-tip with ● 2 x 2 ml, ▲ 2 x 10 ml or ■ 2 x 30 ml Buffer QC. Allow Buffer QC to move through the QIAGEN-tip by gravity flow.
9. Elute DNA with ● 0.8 ml, ▲ 5 ml or ■ 15 ml Buffer QF into a clean ● 2 ml, ▲ 15 ml or ■ 50 ml vessel. For constructs larger than 45 kb, prewarming the elution buffer to 65°C may help to increase the yield.
10. Precipitate DNA by adding ● 0.56 ml, ▲ 3.5 ml or ■ 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.
11. Wash the DNA pellet with ● 1 ml, ▲ 2 ml or ■ 5 ml room-temperature 70% ethanol and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant supernatant.
12. Air-dry pellet for 5–10 min and redissolve DNA in a suitable volume of appropriate buffer (e.g., TE buffer, pH 8.0, or 10 mM Tris·Cl, pH 8.5).

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