Quick-Start Protocol March 2016

HiSpeed® Plasmid Maxi Kit

The HiSpeed Plasmid Maxi Kit (cat. nos. 12662 and 12663) can be stored at room temperature (15–25°C) for at least 2 years if not otherwise stated on label.

Further information

- HiSpeed Plasmid Purification Handbook: **www.qiagen.com/HB-1195**
- 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.

1. Pellet 150 ml (high-copy plasmid) or 250 ml (low-copy plasmid) overnight LB culture at 6000 x *g* for 15 min at 4°C.
2. Resuspend the bacterial pellet homogeneously in 10 ml Buffer P1.
3. Add 10 ml Buffer P2, mix 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 cap onto the outlet nozzle of the QIAfilter Cartridge. Place the QIAfilter Cartridge into a convenient tube or a QIArack (cat. no. 19015).
5. Add 10 ml prechilled Buffer P3, and 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!
7. Equilibrate a HiSpeed Tip with 10 ml Buffer QBT, allowing it to enter the resin.

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 HiSpeed Tip.
9. After the lysate has entered, wash the HiSpeed Tip with 60 ml Buffer QC.
10. Elute DNA with 15 ml Buffer QF.
11. Precipitate DNA by adding 10.5 ml isopropanol, mix and incubate for 5 min.
12. During the incubation, remove the plunger from a 30 ml syringe and attach the QIAprecipitator Module onto the outlet nozzle.
13. Place the QIAprecipitator over a waste bottle, transfer the eluate–isopropanol mixture into the syringe and insert the plunger. Filter the eluate–isopropanol mixture through the QIAprecipitator using constant pressure.
14. Remove the QIAprecipitator from the syringe and pull out the plunger. Re-attach the QIAprecipitator and add 2 ml 70% ethanol to the syringe. Wash the DNA by inserting the plunger and pressing the ethanol through the QIAprecipitator.
15. Remove the QIAprecipitator from the syringe and pull out the plunger. Attach the QIAprecipitator again, insert the plunger and dry the membrane by pressing air through the QIAprecipitator forcefully. Repeat this step several times.
16. Dry the outlet nozzle of the QIAprecipitator with adsorbent paper.
17. Remove the plunger from a new 5 ml syringe, attach the QIAprecipitator and hold the outlet over a 1.5 ml collection tube. Add 1 ml Buffer TE to the 5 ml syringe. Insert the plunger and elute the DNA into the collection tube using constant pressure.
18. Remove the QIAprecipitator from the 5 ml syringe, pull out the plunger and re-attach the QIAprecipitator to the 5 ml syringe.
19. Transfer the eluate from step 17 to the 5 ml syringe and elute for a second time into the same 1.5 ml tube.

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