QIAGEN Supplementary Protocol:

Isolation of endotoxin-free plasmid DNA using the QIAGEN® Plasmid Midi Kit

This protocol is for purification of up to 100 µg endotoxin-free plasmid DNA using QIAGEN-tip 100. Endotoxin-free DNA will improve transfection into sensitive eukaryotic cells and is essential for gene therapy research. Please be sure to read all background information and the protocol notes in the *QIAGEN® Plasmid Purification Handbook* provided with the kit carefully before beginning this procedure.

Please be sure to read the *QIAGEN Plasmid Purification Handbook* and the detailed EndoFree® Plasmid Maxi Protocol carefully before beginning this procedure.

The following kit combinations can be used for Midi scale preparation of endotoxin-free DNA:

Kit combinations

Kit	Cat. No.		Kit	Cat. No.
QIAfilter™ Plasmid Midi Kit (25)	12243	**and**	EndoFree Plasmid Maxi Kit (10)	12362
QIAfilter Plasmid Midi Kit (25)	12243	**and**	EndoFree Plasmid Buffer Set	19048

Spare kit components such as QIAGEN-tip 500 or buffers can be used for other plasmid preparations that are not required to be endotoxin-free.

Maximum recommended culture volumes*

	EndoFree Midi
High-copy plasmids	25 ml
Low-copy plasmids†	50 ml

* *Expected yields are 75–100 µg for high-copy plasmids and 10–50 µg for low copy plasmids, using these culture volumes.*

† *The maximum recommended culture volume applies to the capacity to the QIAfilter Midi Cartridge. If higher yields of low-copy plasmids, within the capacity of QIAGEN-tip100 are desired, the lysates from two QIAfilter Midi Cartridges can be loaded onto one QIAGEN-tip 100.*

Important notes before starting

- Use endotoxin-free plastic pipet tips and tubes for elution and subsequent steps. Endotoxin-free or pyrogen-free plasticware is widely available. Please check with your preferred supplier for recommendations.
- Add RNase A to Buffer P1 to give a final concentration of 100 µg/ml.
- To prepare endotoxin-free 70% ethanol, add 40 ml of 96–100% ethanol to the endotoxin-free water supplied with the kit.
- Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.
- Pre-chill Buffer P3 to 4°C.

Procedure

1. **Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 ml LB medium containing the appropriate selective antibiotic. Incubate for ~8 hours at 37°C with vigorous shaking (~300 rpm).**
2. **Dilute the starter culture 1/500 to 1/1000 into selective LB medium. For high-copy plasmids inoculate 25 ml medium, and for low-copy plasmids, inoculate 50 ml medium. Grow at 37°C for 12–16 hours with vigorous shaking (~300 rpm).**
3. **Harvest the bacterial cells by centrifugation at 6000 x *g* for 15 min at 4°C.**
4. **Resuspend the bacterial pellet in 4 ml Buffer P1.**

   Ensure that RNase A has been added to Buffer P1. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.
5. **Add 4 ml Buffer P2, mix gently but thoroughly by inverting 4–6 times, and incubate at room temperature for 5 min.**

   Do not vortex, as this will result in shearing of genomic DNA. The lysate should appear viscous. Do not allow the lysis reaction to proceed more than 5 min.

   During the incubation prepare the QIAfilter Cartridge:

   Screw the cap onto the outlet nozzle of the QIAfilter Midi Cartridge to prevent dripping. Place the QIAfilter Cartridge into a convenient tube.
6. **Add 4 ml chilled Buffer P3 to the lysate, and mix immediately but gently by inverting 4–6 times. Proceed directly to step 7. Do not incubate the lysate on ice.**
7. **Pour the lysate into the barrel of the QIAfilter Cartridge. Incubate at room temperature for 10 min. Do not insert the plunger.**

   Important: This 10 min incubation at room temperature is essential for optimal performance of the QIAfilter Midi Cartridge. Do not agitate the QIAfilter Cartridge during this time.
8. **Remove the cap from the QIAfilter outlet nozzle. Gently insert the plunger into the QIAfilter Midi Cartridge and filter the cell lysate into a convenient tube.**

9. **Add 1ml (0.1 volumes) Buffer ER to the filtered lysate, mix by inverting the tube approximately 10 times, and incubate on ice for 30 min.**

   After the addition of Buffer ER the lysate appears turbid, but will become clear again during the incubation on ice.

10. **Equilibrate a QIAGEN-tip 100 by applying 4 ml Buffer QBT, and allow the column to empty by gravity flow.**

11. **Apply the cleared lysate from step 9 to the QIAGEN-tip and allow it to enter the resin by gravity flow.**

    Note: All subsequent steps have to be performed with endotoxin free buffers and materials. Please make sure to only use the wash and elution buffers provided with the EndoFree Plasmid Kit or EndoFree Buffer Set.

12. **Wash the QIAGEN-tip with 2 x 10 ml Buffer QC.**

13. **Elute DNA with 5 ml Buffer QN.**

    Collect the eluate in a 10 ml endotoxin-free tube. Use of polycarbonate centrifuge tubes for collection is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

14. **Precipitate DNA by adding 3.5 ml (0.7 volumes) room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at ≥15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant.**

15. **Wash DNA pellet with 2 ml of endotoxin-free room-temperature 70% ethanol and centrifuge at ≥15,000 x *g* for 10 min.**

16. **Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of endotoxin-free Buffer TE, provided with the kit or buffer set.**

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor.
Selected handbooks can be downloaded from **www.qiagen.com/literature/handbooks/default.asp**.
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from **www.qiagen.com/ts/msds.asp**.