QIAGEN Supplementary Protocol:

Removal of endotoxins from purified plasmid DNA using the EndoFree® Plasmid Maxi Kit

Endotoxin-free DNA is essential for gene therapy research and will improve transfection into sensitive eukaryotic cells. For detailed background information on endotoxins, please refer *to the QIAGEN® Plasmid Purification Handbook*.

Throughout this protocol single-underlined text denotes QIAGEN-tip 100 volumes and double-underlined text denotes QIAGEN-tip 500 volumes.

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

Important notes before starting

- Plasmid DNA must be free of SDS and other anionic detergents.
- Use endotoxin-free plastic pipet tips and tubes for elution and subsequent steps. Alternatively, glass tubes may be used if they are baked overnight at 180°C to destroy attached endotoxins.
- Use only the buffers supplied in the QIAGEN EndoFree Plasmid Maxi Kit or the EndoFree Plasmid Buffer Set, which are certified to be endotoxin-free.
- Select the QIAGEN-tip size appropriate for the amount of DNA to be purified: QIAGEN-tip 100 for up to 100 µg plasmid DNA or QIAGEN-tip 500 for up to 500 µg plasmid DNA.

Procedure

1. **Adjust the DNA sample to 750 mM NaCl, 50 mM MOPS, pH 7.0.**
   The final volume should be 5 ml (QIAGEN-tip 100) or 12 ml (QIAGEN-tip 500).
2. **Add 0.5 ml (QIAGEN-tip 100) or 1.2 ml (QIAGEN-tip 500) Buffer ER to the DNA solution.**
3. **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.
4. **Equilibrate a QIAGEN-tip 100 or QIAGEN-tip 500 by applying 4 ml or 10 ml Buffer QBT, and allow the column to empty by gravity flow.**
5. **Apply the DNA solution from step 3 to the QIAGEN-tip and allow it to enter the resin by gravity flow.**
   The presence of Buffer ER may cause the lysate to become turbid again. However, this does not affect the performance of the procedure.

6. **Wash the QIAGEN-tip with 2 x 10 ml or 2 x 30 ml Buffer QC.**

   Important: For all subsequent steps use endotoxin-free plasticware (e.g., new polypropylene centrifuge tubes) or pre-treated glassware.

7. **Elute DNA with 5 ml or 15 ml Buffer QN.**

8. **Precipitate DNA by adding 3.5 ml or 10.5 ml 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.**

9. **Wash DNA pellet with 1 ml or 2.5 ml of endotoxin-free room-temperature 70% ethanol and centrifuge at ≥15,000 x g for 10 min. Carefully decant the supernatant without disturbing the pellet.**

   Add 40 ml of 96–100% ethanol to the endotoxin-free water supplied with the EndoFree Plasmid Maxi Kit or EndoFree Plasmid Buffer Set.

10. **Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of endotoxin-free Buffer TE.**

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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**.