April 2015

EndoFree® Plasmid Purification Handbook

EndoFree Plasmid Maxi, Mega, Giga Kits

For purification of advanced transfection-grade plasmid DNA

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Contents

Kit Contents

EndoFree Plasmid Kit	Maxi (10)	Mega (5)	Giga (5)
Catalog no.	**12362**	**12381**	**12391**
QIAGEN-tip 500	10	–	–
QIAGEN-tip 2500	–	5	–
QIAGEN-tip 10000	–	–	5
QIAfilter Maxi Cartridges	10	–	–
QIAfilter Mega-Giga Cartridges*	–	5	5
Caps for QIAfilter	10	–	–
Buffer P1	110 ml	2 x 140 ml	3 x 250 ml
Buffer P2	110 ml	2 x 140 ml	3 x 250 ml
Buffer P3	110 ml	2 x 140 ml	3 x 250 ml
Buffer FWB2	–	2 x 140 ml	2 x 140 ml
Buffer QBT	2 x 60 ml	200 ml	2 x 200 ml
Buffer QC	3 x 240 ml	5 x 220 ml	7 x 500 ml
Buffer QN	200 ml	200 ml	510 ml
Buffer ER	30 ml	80 ml	200 ml
Buffer TE	30 ml	110 ml	110 ml
Endotoxin-free water for 70% ethanol	17 ml	17 ml	17 ml
LyseBlue®	110 µl	2 x 140 µl	3 x 250 µl
RNase A†	11 mg	2 x 14 mg	3 x 25 mg
Quick-Start Protocol	1	1	1
Certificate of Analysis	1	1	1

* The QIAfilter Mega-Giga Cartridge is designed for use with a 1 liter, 45-mm-neck glass bottle (e.g. Schott, cat. no. 21810154, or Corning, cat. no. 1395-1L). **Note**: Bottle is not included.

† Provided in a 10 mg/ml or 100 mg/ml solution.

Storage

QIAGEN-tips and QIAfilter Cartridges should be stored dry and at room temperature (15–25°C). They can be stored for at least 2 years without showing any reduction in performance, capacity, or quality of separation.

EndoFree Plasmid Kits should be stored at room temperature. After adding RNase A, Buffer P1 should be stored at 2–8°C and is stable for 6 months. Other buffers and RNase A stock solution can be stored for 2 years at room temperature.

Intended Use

EndoFree Plasmid Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Total Quality Management System, each lot of the EndoFree Plasmid Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

EndoFree plasmid purification kits are based on the remarkable selectivity of patented QIAGEN resin, allowing purification of ultrapure supercoiled plasmid DNA with high yields.

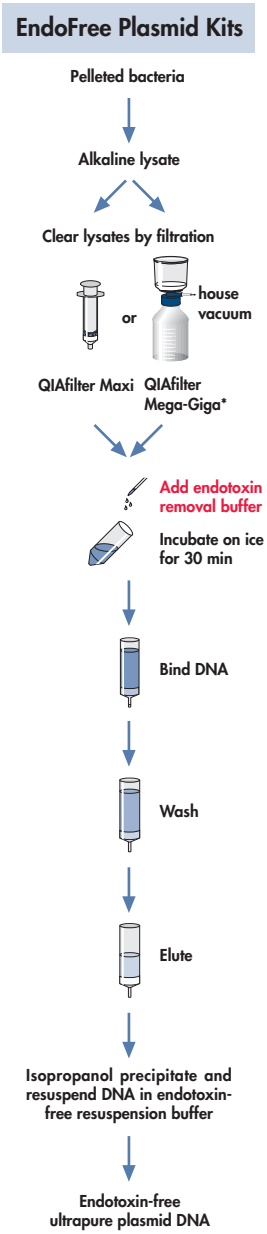
Anion-exchange–based QIAGEN-tips yield transfection-grade DNA, which is highly suited for use in a broad variety of demanding applications such as transfection, in vitro transcription and translation, and enzymatic modifications. QIAGEN offers the most comprehensive portfolio of tailored plasmid purification kits for any scale, throughput, or downstream application. Select the optimum plasmid kit for your requirements by visiting our online selection guide at www.qiagen.com/products/plasmid/selectionguide. For transfection, QIAGEN also offers the advanced PolyFect®, SuperFect®, and Effectene® transfection reagents. These reagents, combined with the high-quality plasmid DNA obtained from QIAGEN, QIAfilter, HiSpeed®, and EndoFree Plasmid Kits, provide optimal transfection results (for ordering information, see page 42).

Principle and procedure

QIAGEN plasmid purification protocols are based on a modified alkaline lysis procedure, followed by binding of plasmid DNA to QIAGEN resin under appropriate low-salt and pH conditions. RNA, proteins, dyes, and low-molecular-weight impurities are removed by a medium-salt wash. Plasmid DNA is eluted in a high-salt buffer and then concentrated and desalted by isopropanol precipitation.

Each disposable QIAGEN-tip packed with QIAGEN resin is designed to operate by gravity flow, reducing the amount of hands-on time required for the purification procedure.

QIAGEN-tips are highly suited for rapid and simple preparation of multiple samples, while QIAfilter Cartridges provided in EndoFree Plasmid Kits enable quick and efficient clearing of bacterial lysates without centrifugation. QIAfilter Midi and Maxi Cartridges have a syringe format and lysates are cleared by pushing the liquid through the filter (Figure 2A, page 12). QIAfilter Mega-Giga Cartridges are special filter units which operate with any vacuum source to clear bacterial lysates from up to 2.5 liters of bacterial culture (Figure 2B, page 12). QIAfilter Midi, Maxi, and Mega-Giga Cartridges completely remove SDS precipitates for efficient clearing in a fraction of the time needed for conventional centrifugation. Plasmid DNA from the filtered lysate is then efficiently purified using a QIAGEN-tip.



* Bottle not included.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

For all protocols:

- Standard microbiological equipment for growing and harvesting bacteria (e.g., inoculating loop, culture tubes and flasks, 37°C shaking incubator, and centrifuge with rotor and tubes or bottles for harvesting cells)
- QIArack or equivalent holder (see "Setup of QIAGEN-tips", page 11)
- Isopropanol
- Ethanol

For Mega and Giga Kit protocols:

- Vacuum source and bottle as specified in the "Important points before starting" section, page 20.

Important Notes

Please take a few moments to read this handbook carefully before beginning the DNA preparation. If QIAGEN plasmid purification kits are new to you, please visit our plasmid resource page at www.qiagen.com/goto/plasmidinfo and click on the link "General Considerations for Optimal Results". Also be sure to read and follow the appropriate detailed protocol.

Plasmid size

Plasmids and cosmids up to 50 kb in size can be purified using EndoFree Plasmid Kits. For larger constructs, QIAGEN Plasmid Kits or the Large-Construct Kit are recommended.

Plasmid/cosmid copy number

Plasmid and cosmids vary in copy number, depending on the origin of replication they contain, their size, and the size of insert. The protocols in this handbook are grouped according to the copy number of the plasmid or cosmid to be purified. For more details, visit our plasmid resource page at www.qiagen.com/goto/plasmidinfo and click on the link "General Considerations for Optimal Results".

Host strains

The strain used to propagate a plasmid can have a substantial influence on quality of the purified DNA. Host strains such as DH1, DH5®α, and C600 yield high-quality DNA with QIAGEN protocols. The slower growing strain XL1-Blue also yields DNA of very high quality.

Strain HB101 and its derivatives, such as TG1 and the JM100 series, contain large amounts of carbohydrates that are released during lysis and can inhibit enzyme activities if not completely removed. In addition, some strains, such as JM101, JM110, and HB101, have high levels of endonuclease activity and yield DNA of lower quality. If the quality of purified DNA is not as expected, a change of host strain should be considered. If difficulty is encountered with strains such as TG1 and Top10F, we recommend either reducing the amount of culture volume or doubling the volumes of Buffers P1, P2, and P3 to improve the ratio of biomass to lysis buffers for optimized lysis conditions.

Table 1. Origins of replication and copy numbers of various plasmids and cosmids

DNA construct	Origin of replication	Copy number	Classification
Plasmids			
pUC vectors	pMB1*	500–700	High copy
pBluescript® vectors	ColE1	300–500	High copy
pGEM® vectors	pMB1*	300–400	High copy
pTZ vectors	pMB1*	>1000	High copy
pBR322 and derivatives	pMB1*	15–20	Low copy
pACYC and derivatives	p15A	10–12	Low copy
pSC101 and derivatives	pSC101	~5	Very low copy
Cosmids			
SuperCos	ColE1	10–20	Low copy
pWE15	ColE1	10–20	Low copy

* The pMB1 origin of replication is closely related to that of ColE1 and falls in the same incompatibility group. The high-copy plasmids listed here contain mutated versions of this origin.

Culture media

QIAGEN plasmid purification protocols are optimized for use with cultures grown in standard Luria Bertani (LB) medium to a cell density of approximately 3–4 x 10^9 cells/ml, which typically corresponds to a pellet wet weight of approximately 3 g/liter medium. Please note that a number of slightly different LB culture broths, containing different concentrations of NaCl, are commonly used. We recommend growing cultures in LB medium containing 10 g NaCl per liter (Table 2) to obtain the highest plasmid yields.

Rich media are not recommended for plasmid preparation with QIAGEN-tips. If rich media must be used, growth time must be optimized, and culture volumes reduced. For more details, visit our plasmid resource page at www.qiagen.com/goto/plasmidinfo and click on the link "General Considerations for Optimal Results".

Table 2. Composition of Luria Bertani medium

Contents	Per liter
Tryptone	10 g
Yeast extract	5 g
NaCl	10 g

Please refer to Appendix C on page 40 for preparation of LB medium.

Culture volume

Do not exceed the maximum recommended culture volumes given at the beginning of each protocol (and on the card inside the back cover of this handbook). Using larger culture volumes will lead to an increase in biomass and can affect the efficiency of alkaline lysis, leading to reduced yield and purity of the preparation.

The protocol for EndoFree Plasmid Kits is optimized for use with cultures grown in standard Luria Bertani (LB) medium (see page 10), grown to a cell density of approximately 3–4 x 10^9 cells per ml. We advise harvesting cultures after approximately 12–16 hours of growth, which typically is the transition from logarithmic into stationary growth phase. It is best to assess the cell density of the culture and, if that is too high, to reduce the culture volumes accordingly or increase the volumes of Buffers P1, P2, and P3. A high ratio of biomass to lysis buffers will result in poor lysis conditions and subsequently low DNA yield and purity. For determination of cell density, calibration of each individual spectrophotometer is required to facilitate accurate conversion of OD_{600} measurements into the number of cells per milliliter. This can be achieved by plating serial dilutions of a culture onto LB-agar plates in the absence of antibiotics. The counted colonies are used to calculate the number of cells per milliliter, which is then set in relation to the measured OD_{600} values.

Capacity of QIAGEN-tips

QIAGEN-tips are available in a variety of sizes for preparation of as little as 20 µg or as much as 10 mg plasmid DNA. The maximum plasmid binding capacities of the QIAGEN-tips 20, 100, 500, 2500, and 10000 are at least 20 µg, 100 µg, 500 µg, 2.5 mg, and 10 mg, respectively. Actual yields will depend on culture volume, culture medium, plasmid copy number size of insert, and host strain. For more details, visit our plasmid resource page at www.qiagen.com/goto/plasmidinfo and click on the link "General Considerations for Optimal Results".

Setup of QIAGEN-tips

QIAGEN-tips may be held upright in a suitable collection vessel such as a tube or flask, using the tip holders provided with the kits (Figure 1A). Alternatively, the QIAGEN-tips 20, 100, 500, and 2500 may be placed in the QIArack (cat. no. 19015) (Figures 1B and 2A).

Analytical gel analysis

The success of the plasmid purification procedure can be monitored on an analytical gel (see Figure 3, page 35). We recommend removing and saving aliquots where indicated during the purification procedure (samples 1–4). If the plasmid DNA is of low yield or quality, the samples can be analyzed by agarose gel electrophoresis to determine the stage of the purification where the problem occurred (see page 34).

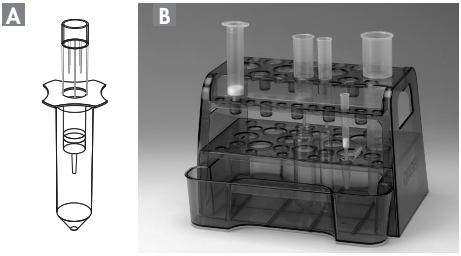


Figure 1. Setup of QIAGEN-tips with A tip holder or B with the QIArack.

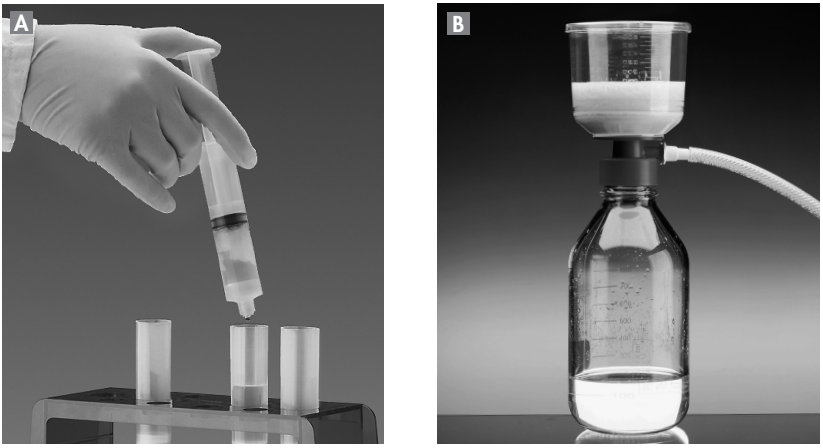


Figure 2. A The syringe-format QIAfilter Maxi Cartridge in use with QIAGEN-tips in the QIArack. B The vacuum-operated QIAfilter Mega-Giga Cartridge in use. Note that the bottle is not included in kits.

Convenient stopping points in protocols

For all protocols, the purification procedure can be stopped and continued later by freezing the cell pellets obtained by centrifugation. The frozen cell pellets can be stored at –20 °C for several weeks. In addition, the DNA eluted from the QIAGEN-tip can be stored overnight at 2–8°C,* after which the protocol can be continued. These stopping points are indicated by the symbol ⊗.

* Longer storage is not recommended.

Using LyseBlue reagent

LyseBlue is a color indicator that provides visual identification of optimum buffer mixing. This prevents common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. This makes LyseBlue ideal for use by researchers who have not had much experience with plasmid preparations as well as experienced scientists who want to be assured of maximum product yield.

LyseBlue can be added to the resuspension buffer (Buffer P1) bottle before use. Alternatively, smaller amounts of LyseBlue can be added to aliquots of Buffer P1, enabling single plasmid preparations incorporating visual lysis control to be performed.

LyseBlue reagent should be added to Buffer P1 at a ratio of 1:1000 to achieve the required working concentration (e.g., 10 µl LyseBlue into 10 ml Buffer P1). Make sufficient LyseBlue/Buffer P1 working solution for the number of plasmid preps being performed.

LyseBlue precipitates after addition into Buffer P1. This precipitate will completely dissolve after addition of Buffer P2. Shake Buffer P1 before use to resuspend LyseBlue particles.

The plasmid preparation procedure is performed as usual. After addition of Buffer P2 to Buffer P1, the color of the suspension changes to blue. Mixing should result in a homogeneously colored suspension. If the suspension contains localized regions of colorless solution or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

Upon addition of neutralization buffer (Buffer P3), LyseBlue turns colorless. The presence of a homogeneous solution with no traces of blue indicates that SDS from the lysis buffer has been effectively precipitated.

Protocol: Plasmid or Cosmid DNA Purification using the EndoFree Plasmid Maxi Kit

This protocol is designed for purification of up to 500 µg endotoxin-free plasmid DNA using the EndoFree Plasmid Maxi Kit. Endotoxin-free DNA will improve transfection into sensitive eukaryotic cells and is essential for gene therapy research. For background information on endotoxins, see pages 36–38. Low-copy plasmids which have been amplified in the presence of chloramphenicol should be treated as high-copy plasmids when choosing the appropriate culture volume.

Table 3. Maximum recommended culture volumes*

	EndoFree Maxi
High-copy plasmids	100 ml
Low-copy plasmids†	250 ml

* Expected yields are 300–500 µg for high-copy plasmids and 50–250 µg for low-copy plasmids, using these culture volumes.

† The maximum culture volume recommended applies to the capacity of the QIAfilter Maxi Cartridge. If higher yields of low-copy plasmids yields are desired, the lysates from two QIAfilter Maxi Cartridges can be loaded onto one QIAGEN-tip 500.

Important points before starting

- New users are advised to familiarize themselves with the detailed protocol provided in this handbook. In addition, extensive background information is provided on our plasmid resource page www.qiagen.com/goto/plasmidinfo.
- If working with low-copy vectors, it may be beneficial to increase the lysis buffer volumes to increase the efficiency of alkaline lysis, and thereby the DNA yield. In case additional Buffers P1, P2, and P3 are needed, their compositions are provided in Appendix C, on page 40. Alternatively, the buffers and additional QIAfilter Cartridges may be purchased separately.
- Use endotoxin-free or pyrogen-free plastic pipet tips and tubes for elution and subsequent steps (step 13 onwards). Endotoxin-free or pyrogen-free plasticware can be obtained from many common vendors. Please check with your current supplier to obtain recommendations. Alternatively, glass tubes may be used if they are baked overnight at 180°C to destroy endotoxins.
- In contrast to the standard protocol there is no incubation on ice after addition of Buffer P3.
- **Optional**: Remove samples at the indicated steps to monitor the procedure on an analytical gel (see page 34).

Things to do before starting

- Add the provided RNase A solution to Buffer P1 before use. Use 1 vial RNase A (centrifuge briefly before use) per bottle Buffer P1 for 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 at 4°C.
- **Optional**: Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use 1 vial LyseBlue reagent per bottle Buffer P1 for a final dilution of 1:1000 (e.g., 10 µl LyseBlue into 10 ml Buffer P1). LyseBlue provides visual identification of optimum buffer mixing, thereby preventing the common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. For more details see "Using LyseBlue reagent" on page 13.

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 approx. 8 h at 37°C with vigorous shaking (approx. 300 rpm).**

   Use a tube or flask with a volume of at least 4 times the volume of the culture.

2. **Dilute the starter culture 1/500 to 1/1000 into selective LB medium. For high-copy plasmids, inoculate 100 ml medium with 100–200 µl of starter culture. For low-copy plasmids, inoculate 250 ml medium with 250–500 µl of starter culture. Grow at 37°C for 12–16 h with vigorous shaking (approx. 300 rpm).**

   Use a flask or vessel with a volume of at least 4 times the volume of the culture. The culture should reach a cell density of approximately 3–4 x 10^9 cells per milliliter, which typically corresponds to a pellet wet weight of approximately 3 g/liter medium (see page 11).

3. **Harvest the bacterial cells by centrifugation at 6000 x *g* for 15 min at 4°C.**

   ⊗ If you wish to stop the protocol and continue later, freeze the cell pellets at –20 °C.

4. **Resuspend the bacterial pellet in 10 ml Buffer P1.**

   For efficient lysis it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. Ensure that RNase A has been added to Buffer P1.

   If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle before use to ensure LyseBlue particles are completely resuspended.

   The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

5. **Add 10 ml Buffer P2, mix thoroughly by vigorously inverting the sealed tube 4–6 times, and incubate at room temperature (15–25°C) 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 for more than 5 min. After use, the bottle containing Buffer P2 should be closed immediately to avoid acidification from CO_2 in the air.

   If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

   During the incubation prepare the QIAfilter Cartridge:

   Screw the cap onto the outlet nozzle of the QIAfilter Maxi Cartridge.

   Place the QIAfilter Cartridge in a convenient tube.

6. **Add 10 ml chilled Buffer P3 to the lysate and mix immediately and thoroughly by vigorously inverting 4–6 times. Proceed directly to step 7. Do not incubate the lysate on ice.**

   Precipitation is enhanced by using chilled Buffer P3. After addition of Buffer P3, a fluffy white material forms and the lysate becomes less viscous. The precipitated material contains genomic DNA, proteins, cell debris, and KDS. The lysate should be mixed thoroughly to ensure even potassium dodecyl sulfate precipitation. If the mixture still appears viscous, more mixing is required to completely neutralize the solution. It is important to transfer the lysate into the QIAfilter Cartridge immediately to prevent later disruption of the precipitate layer.

   If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

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 Maxi Cartridge. Do not agitate the QIAfilter Cartridge during this time. A precipitate containing proteins, genomic DNA, and detergent will float and form a layer on top of the solution. This ensures convenient filtration without clogging. If, after the 10 min incubation, the precipitate has not floated to the top of the solution, carefully run a sterile pipet tip around the walls of the cartridge to dislodge it.

8. **Remove the cap from the QIAfilter Cartridge outlet nozzle. Gently insert the plunger into the QIAfilter Maxi Cartridge and filter the cell lysate into a 50 ml tube.**

   Filter until all of the lysate has passed through the QIAfilter Cartridge, but do not apply extreme force. Approximately 25 ml of the lysate is generally recovered after filtration.

   Optional: Remove a 120 µl sample of the filtered lysate and save for an analytical gel (sample 1) to determine whether growth and lysis conditions were optimal.

9. **Add 2.5 ml 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 500 by applying 10 ml Buffer QBT, and allow the column to empty by gravity flow.**

    Flow of buffer will begin automatically by reduction in surface tension due to the presence of detergent in the equilibration buffer. Allow the QIAGEN-tip to drain completely. QIAGEN-tips can be left unattended, since the flow of buffer will stop when the meniscus reaches the upper frit in the column.

11. **Apply the filtered lysate from step 9 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.

    Optional: Remove a 120 µl sample of the flow-through and save for an analytical gel (sample 2) to determine the efficiency of DNA binding to the QIAGEN resin.

12. Wash the QIAGEN-tip with 2 x 30 ml Buffer QC.

Allow Buffer QC to move through the QIAGEN-tip by gravity flow. The first wash is sufficient to remove contaminants in the majority of plasmid preparations. The second wash is particularly necessary when large culture volumes or bacterial strains containing large amounts of carbohydrates are used.

Optional: Remove a 240 µl sample from the combined wash fractions and save for an analytical gel (sample 3).

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

13. Elute DNA with 15 ml Buffer QN.

Collect the eluate in a 30 ml endotoxin-free or pyrogen-free tube. Use of polycarbonate centrifuge tubes for collection is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

Note: For constructs larger than 45–50 kb, prewarming the elution buffer to 65°C may help to increase yield.

Optional: Remove a 60 µl sample of the eluate and save for an analytical gel (sample 4).

⊗ If you wish to stop the protocol and continue later, store the eluate at 4°C. Storage periods longer than overnight are not recommended.

14. Precipitate DNA by adding 10.5 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.

All solutions should be at room temperature to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. Alternatively, disposable conical bottom centrifuge tubes can be used for centrifugation at 5000 x *g* for 60 min at 4°C. Isopropanol pellets have a glassy appearance and may be more difficult to see than the fluffy, salt-containing pellets that result from ethanol precipitation. Marking the outside of the tube before centrifugation allows the pellet to be more easily located. Isopropanol pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant.

15. Wash DNA pellet with 5 ml of endotoxin-free room-temperature 70% ethanol (add 40 ml of 96–100% ethanol to the endotoxin-free water supplied with the kit) and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.

Alternatively, disposable conical-bottom centrifuge tubes can be used for centrifugation at 5000 x *g* for 60 min at 4°C. The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

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

    Redissolve DNA pellet by rinsing the walls to recover the DNA, especially if glass tubes have been used. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided. Overdrying the pellet will make the DNA difficult to redissolve. DNA dissolves best under alkaline conditions; it does not easily dissolve in acidic buffers.

Determination of yield

To determine the yield, DNA concentration should be determined by both UV spectrophotometry at 260 nm and quantitative analysis on an agarose gel. For reliable spectrophotometric DNA quantification, A_{260} readings should lie between 0.1 and 1.0.

Agarose gel analysis

We recommend removing and saving aliquots during the purification procedure (samples 1–4). If the plasmid DNA is of low yield or quality, the samples can be analyzed by agarose gel electrophoresis to determine the stage of the purification procedure where the problem occurred (see page 34).

Protocol: Plasmid or Cosmid DNA Purification using EndoFree Plasmid Mega and Giga Kits

This protocol is designed for preparation of up to 2.5 mg of high- or low-copy plasmid and cosmid DNA using the EndoFree Plasmid Mega Kit, or up to 10 mg of high-copy plasmid DNA using the EndoFree Plasmid Giga Kit. Endotoxin-free DNA will improve transfection into sensitive eukaryotic cells and is essential for gene therapy research. For background information on endotoxins, see pages 36–38. (Please note: the EndoFree Plasmid Giga Kit is not recommended for low-copy plasmids or cosmids.) Low-copy plasmids which have been amplified in the presence of chloramphenicol should be treated as high-copy plasmids when choosing the appropriate culture volume.

Table 4. Maximum recommended culture volumes*

	EndoFree Mega	EndoFree Giga
High-copy plasmids	500 ml LB culture (1.5 g pellet wet weight)†	2.5 liters LB culture (7.5 g pellet wet weight)†
Low-copy plasmids	2.5 liters LB culture (7.5 g pellet wet weight)†	Not recommended for low-copy plasmids or cosmids‡

* For high-copy plasmids, expected yields are 1.5–2.5 mg for the EndoFree Plasmid Mega Kit and 7.5–10 mg for the EndoFree Plasmid Giga Kit. For low-copy plasmids, expected yields are 0.5–2.5 mg for the EndoFree Plasmid Mega Kit. The EndoFree Plasmid Giga Kit is not recommended for low-copy plasmid preparations.

† On average, a healthy 1 liter shaker culture yields a pellet with a wet weight of approximately 3 g. When working with fermentation cultures, however, the pellet wet weight may be significantly higher. Therefore, when using fermentation cultures please refer to the pellet wet weight instead of the recommended culture volumes.

‡ Due to the large culture volume required for preparation of low-copy plasmid and cosmid DNA and the limited capacity of the QIAfilter Mega-Giga Cartridge, the EndoFree Plasmid Mega Kit is a better choice than the EndoFree Plasmid Giga Kit for purification of low-copy plasmids and cosmids.

Important points before starting

- New users are advised to familiarize themselves with the detailed protocol provided in this handbook. In addition, extensive background information is provided on our plasmid resource page www.qiagen.com/goto/plasmidinfo.
- If working with low-copy vectors, it may be beneficial to increase the lysis buffer volumes to increase the efficiency of alkaline lysis, and thereby the DNA yield. In case additional Buffers P1, P2, and P3 are needed, their compositions are provided in Appendix C, on page 40. Alternatively, the buffers and additional QIAfilter Cartridges may be purchased separately.

- Use endotoxin-free or pyrogen-free plastic pipet tips and tubes for elution and subsequent steps (step 15 onwards). Endotoxin-free or pyrogen-free plasticware can be obtained from many common vendors. Please check with your current supplier to obtain recommendations. Alternatively, glass tubes may be used if they are baked overnight at 180°C to destroy endotoxins.
- The QIAfilter Mega-Giga Cartridge is designed for use with a 1 liter, 45 mm-neck, vacuum-resistant glass bottle (e.g., Schott, cat. no. 2181054, or Corning, cat. no. 1395-1L). **Note**: Bottles are not included in the kit and must be supplied by the user. The cartridge operates with any vacuum source (e.g., a house vacuum, vacuum pump, or water aspirator) that generates vacuum pressures between –200 and –600 millibars (–150 and –450 mm Hg). The vacuum pressure is measured as differential pressure between the inside of the bottle and the atmosphere (1013 millibars or 760 mm Hg). Vacuum recommendations are given in negative units to indicate the required reduction in pressure with respect to the atmosphere.
- To avoid the possibility of implosion, do not use plastic/glass bottles or any other vessels that are not designed for use with a vacuum. Do not use plastic/glass bottles or any other vessels that are cracked or scratched. Wear safety glasses when working near a bottle under vacuum.
- **Optional**: Remove samples at the indicated steps to monitor the procedure on an analytical gel (see page 34).
- In contrast to the standard protocol, the lysate is not incubated on ice after addition of Buffer P3.
- Blue (marked with a ▲) denotes values for the EndoFree Plasmid Mega Kit; red (marked with a ●) denotes values for the EndoFree Plasmid Giga Kit.

Things to do before starting

- Add the provided RNase A solution to Buffer P1 before use. Use 1 vial RNase A (centrifuge briefly before use) per bottle Buffer P1 for 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 at 4°C.

- **Optional**: Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use 1 vial LyseBlue reagent per bottle Buffer P1 for a final dilution of 1:1000 (e.g., 10 µl LyseBlue into 10 ml Buffer P1). LyseBlue provides visual identification of optimum buffer mixing, thereby preventing the common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. For more details see "Using LyseBlue reagent" on page 13.

Procedure

1. **Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 5–10 ml LB medium containing the appropriate selective antibiotic. Incubate for approx. 8 h at 37°C with vigorous shaking (approx. 300 rpm).**

   Use a flask with a volume of at least 4 times the volume of the culture.

2. **Dilute the starter culture 1/500 to 1/1000 into selective LB medium. For high-copy plasmids, inoculate ▲ 500 ml or ● 2.5 liters medium with ▲ 500–1000 µl or ● 2.5–5 ml of starter culture. For low-copy plasmids, inoculate ▲ 2.5 liters medium with ● 2.5–5 ml of starter culture. Grow at 37°C for 12–16 h with vigorous shaking (approx. 300 rpm).**

   Use a flask or vessel with a volume of at least 4 times the volume of the culture. The culture should reach a cell density of approximately 3–4 x 10^9 cells per milliliter, which typically corresponds to a pellet wet weight of approximately 3 g/liter medium.

3. **Harvest the bacterial cells by centrifugation at 6000 x *g* for 15 min at 4°C.**

   ⊗ If you wish to stop the protocol and continue later, freeze the cell pellets at –20 °C.

4. **Screw the QIAfilter Mega-Giga Cartridge onto a 45 mm-neck glass bottle and connect it to a vacuum source.**

   Do not overtighten the QIAfilter Cartridge on the bottle neck, because the QIAfilter Cartridge plastic may crack.

5. **Resuspend the bacterial pellet in ▲ 50 ml or ● 125 ml of Buffer P1.**

   For efficient lysis it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. We recommend a 500 ml bottle for Mega preparations and a 1000 ml bottle for Giga preparations. Ensure that the RNase A has been added to Buffer P1.

   If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle before use to ensure LyseBlue particles are completely resuspended.

   The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

6. **Add ▲ 50 ml or ● 125 ml of Buffer P2, mix thoroughly by vigorously inverting 4–6 times, and incubate at room temperature (15–25°C) 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 for more than 5 min. After use, the bottle containing Buffer P2 should be closed immediately to avoid acidification of Buffer P2 from CO_2 in the air.

   If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

7. **Add ▲ 50 ml or ● 125 ml chilled Buffer P3 and mix thoroughly by vigorously inverting 4–6 times. Mix well until white, fluffy material has formed and the lysate is no longer viscous. Proceed directly to step 8. Do not incubate on ice.**

   Precipitation is enhanced by using chilled Buffer P3. After addition of Buffer P3, a fluffy, white precipitate containing genomic DNA, proteins, cell debris, and KDS becomes visible. The lysate should be mixed well to reduce the viscosity and prevent clogging of the QIAfilter Cartridge.

   If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

8. **Pour the lysate into the QIAfilter Mega-Giga Cartridge and incubate at room temperature for 10 min.**

   Important: This 10 min incubation at room temperature is essential for optimal performance of the QIAfilter Mega-Giga Cartridge. Do not agitate the QIAfilter Cartridge during this time. A precipitate containing proteins, genomic DNA, and detergent will float and form a layer on top of the solution. This ensures convenient filtration without clogging.

9. **Switch on the vacuum source. After all liquid has been pulled through, switch off the vacuum source. Leave the QIAfilter Cartridge attached.**

10. **Add 50 ml (both ▲ Mega and ● Giga) Buffer FWB2 to the QIAfilter Cartridge and gently stir the precipitate using a sterile spatula. Switch on the vacuum source until the liquid has been pulled through completely.**

    Gentle stirring of the precipitate enhances the flow of liquid through the filter unit. Take care not to disperse the precipitate, as this may result in carryover of cell debris and KDS, which will affect flow and binding characteristics of the QIAGEN column. The filtered lysate in the bottle contains the plasmid DNA.

    Optional: Remove a ▲ 120 µl or ● 75 µl sample from the cleared lysate and save for an analytical gel (sample 1) to determine whether growth and lysis conditions were optimal.

11. **Add ▲ 12.5 ml or ● 30 ml Buffer ER to the filtered lysate, mix by inverting the bottle approximately 10 times, and incubate on ice for 30 min.**

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

12. **Equilibrate a ▲ QIAGEN-tip 2500 or ● QIAGEN-tip 10000 by applying ▲ 35 ml or ● 75 ml Buffer QBT, and allow the column to empty by gravity flow.**

    Flow of buffer will begin automatically by reduction in surface tension due to the presence of detergent in the equilibration buffer. Allow the QIAGEN-tip to drain completely. QIAGEN-tips can be left unattended, since the flow of buffer will stop when the meniscus reaches the upper frit in the column.

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

    Due to the presence of Buffer ER the lysate may become turbid again, however this does not affect the performance of the procedure.

    Optional: Remove a ▲ 120 µl or ● 75 µl sample from the flow-through and save for an analytical gel (sample 2) to determine efficiency of DNA binding to the QIAGEN resin.

14. **Wash the QIAGEN-tip with a total of ▲ 200 ml or a total of ● 600 ml Buffer QC.**

    Allow Buffer QC to move through the QIAGEN-tip by gravity flow. The first half of the volume of wash buffer is sufficient to remove contaminants in the majority of plasmid DNA preparations. The second half is particularly necessary when large culture volumes or bacterial strains producing large amounts of carbohydrates are used.

    Optional: Remove a ▲ **160 µl** or ● 120 µl sample from the combined wash fractions and save for an analytical gel (sample 3).

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

15. **Elute DNA with ▲ 35 ml or ● 100 ml Buffer QN.**

    Use of polycarbonate centrifuge tubes for collection is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

    Optional: Remove a ▲ 22 µl or ● 20 µl sample of the eluate and save for an analytical gel (sample 4).

    Note: For constructs larger than 45–50 kb, prewarming the elution buffer to 65°C may help to increase yield.

    ⊗ If you wish to stop the protocol and continue later, store the eluate at 4°C. Storage periods longer than overnight are not recommended.

16. **Precipitate DNA by adding ▲ 24.5 ml or ● 70 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.**

    All solutions should be at room temperature to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. Alternatively, disposable conical bottom centrifuge tubes can be used for centrifugation at 5000 x *g* for 60 min at 4°C. Isopropanol pellets have a glassy appearance and may be more difficult to see than the fluffy, salt-containing pellets that result from ethanol precipitation. Marking the outside of the tube before centrifugation allows the pellet to be more easily located. Isopropanol pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant.

17. **Wash DNA pellet with ▲ 7 ml or ● 10 ml of endotoxin-free room-temperature 70% ethanol (add 40 ml of 96–100% ethanol to the endotoxin-free water supplied with the kit) and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.**

    Alternatively, disposable conical-bottom centrifuge tubes can be used for centrifugation at 5000 x *g* for 60 min at 4°C. The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

18. **Air-dry the pellet for 10–20 min, and redissolve the DNA in a suitable volume of endotoxin-free Buffer TE.**

    Redissolve the DNA pellet by rinsing the walls to recover the DNA, especially if glass tubes have been used. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided. Overdrying the pellet will make the DNA difficult to redissolve. DNA dissolves best under slightly alkaline conditions; it does not easily dissolve in acidic buffers.

Determination of yield

To determine the yield, DNA concentration should be determined by both UV spectrophotometry at 260 nm and quantitative analysis on an agarose gel. For reliable spectrophotometric DNA quantification, A_{260} readings should lie between 0.1 and 1.0.

Agarose gel analysis

We recommend removing and saving aliquots during the purification procedure (samples 1–4). If the plasmid DNA is of low yield or quality, the samples can be analyzed by agarose gel electrophoresis to determine the stage of the purification procedure where the problem occurred (see page 34).

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
Low or no DNA yield	
No DNA in lysate (sample 1)	
a) Plasmid did not propagate	Please read "Growth of Bacterial Cultures" on our Web page www.qiagen.com/goto/plasmidinfo, and check that the conditions for optimal growth were met.
b) Alkaline lysis was inefficient	If cells have grown to very high densities or a larger amount of cultured medium than recommended was used, the ratio of biomass to lysis reagent is shifted. This may result in poor lysis conditions, because the volumes of Buffers P1, P2, and P3 are not sufficient for setting the plasmid DNA free efficiently. Reduce culture volume or increase volumes of Buffers P1, P2, and P3. Also, insufficient mixing of lysis reagents will result in reduced yield. Mix thoroughly after addition of Buffers P1, P2, and P3 to achieve homogeneous suspensions. Use LyseBlue to visualize efficiency of mixing.
c) Insufficient lysis for low-copy plasmids	For low copy-plasmid preparations, doubling the volumes of lysis buffers P1, P2, and P3 may help to increase plasmid yield and quality (see page 9 and background on our Web page www.qiagen.com/ goto/plasmidinfo).

		Comments and suggestions
d)	Lysate incorrectly prepared	Check Buffer P2 for SDS precipitation resulting from low storage temperatures and dissolve the SDS by warming. The bottle containing Buffer P2 should always be closed immediately after use. Lysis buffers prepared in the laboratory should be prepared according to the instructions on page 40. If necessary, prepare fresh Buffers P1, P2, and P3.

DNA in flow-though fraction (sample 2)

a)	Column was overloaded	Check the culture volume and yield against the capacity of the QIAGEN-tip, as detailed at the beginning of each protocol. Reduce the culture volume accordingly, or select a larger QIAGEN-tip if a higher yield is desired. For very low-copy-number plasmid and cosmid preps requiring very large culture volumes, please see www.qiagen.com/plasmidinfo.
b)	SDS (or other ionic detergent) was in lysate	Chill Buffer P3 before use. If the lysate is cleared by centrifugation, load onto QIAGEN-tip promptly after centrifugation. If lysate is too viscous for effective mixing of Buffer P3, reduce culture volume or increase volumes of Buffers P1, P2, and P3. Use LyseBlue to visualize efficiency of mixing.
c)	Inappropriate salt or pH conditions in buffers	Ensure that any buffers prepared in the laboratory were prepared according to the instructions provided on page 40.
d)	Column flow was uneven	Store QIAGEN-tips at room temperature (15–25°C). If stored under cold, damp conditions for prolonged periods of time, the resin may clump. This problem can be overcome by shaking the column before use.

DNA in Buffer QC wash fraction (sample 3)

a)	Column was overloaded	Check the culture volume and yield against the capacity of the QIAGEN-tip, as detailed at the beginning of each protocol. Reduce the culture volume accordingly, or select a larger QIAGEN-tip if a higher yield is desired. For very low-copy-number plasmid and cosmid preps requiring very large culture volumes, please see www.qiagen.com/plasmidinfo.

		Comments and suggestions
b)	Buffer QC was incorrect	Check pH and salt concentration of Buffer QC. Recover DNA by precipitation, and purify on a new QIAGEN-tip as detailed in "Purification of Plasmid DNA Prepared by Other Methods" on our Web page www.qiagen.com/goto/plasmidinfo.

No DNA in eluate (sample 4)

a)	No DNA in the lysate	See section "No DNA in lysate" page 26.
b)	Elution Buffer QF or QN was incorrect	Check pH and salt concentration of Buffer QF or QN. Recover DNA by eluting with fresh buffer.
c)	DNA passed through in the flow-through or wash fraction	See previous two sections.

Little or no DNA after precipitation

a)	DNA failed to precipitate	Ensure that the precipitate is centrifuged at ≥15,000 x *g* for 30 min. Recover DNA by centrifuging for longer and at higher speeds. Try another isopropanol batch.
b)	DNA pellet was lost	Isopropanol pellets are glassy and may be difficult to see. Mark the outside of the tube before centrifugation. Isopropanol pellets may also be loosely attached to the side of the tube, so pour supernatant off gently.
c)	DNA was poorly redissolved	Check that DNA is completely redissolved. Be sure to wash any DNA off the walls, particularly if glass tubes and a fixed-angle rotor are used. Up to half of the total DNA may be smeared on the walls. Alternatively, a swinging bucket rotor can be used to ensure that the pellet is located at the bottom of the tube.

Plasmid DNA difficult to redissolve

a)	Pellet was overdried	Air-dry pellet instead of using a vacuum, especially if the DNA is of high molecular weight. Redissolve DNA by warming the solution slightly, and allowing more time for redissolving.
b)	Residual isopropanol in pellet	Ensure that pellets are washed with 70% ethanol to remove traces of isopropanol. Redissolve DNA by warming the solution slightly, and allowing more time for redissolving. Increase volume of buffer used for redissolving if necessary.

		Comments and suggestions
c)	Too much salt in pellet	Ensure that isopropanol is at room temperature for precipitation, and wash the pellet twice with room temperature 70% ethanol. Recover DNA by increasing the volume of buffer used for redissolving.
d)	Buffer pH was too low	Ensure that the pH of the buffer used for redissolving is ≥8.0, since DNA does not dissolve well in acidic solutions.
e)	Resuspension volume too low	Increase resuspension volume if the solution above the pellet is highly viscous.

Contaminated DNA/poor-quality DNA

a)	Genomic DNA in the eluate	Mixing of bacterial lysate was too vigorous. The lysate should not be vortexed after addition of Buffers P2 and P3 to prevent shearing of chromosomal DNA. Reduce culture volume if lysate is too viscous for gentle mixing.
b)	RNA in the eluate	RNase A digestion was insufficient. Check culture volume against recommended volumes, and reduce if necessary. Check that the RNase A provided with the kit has been used. If Buffer P1 is more than 6 months old, add more RNase A. Recover DNA by precipitating the eluate, digesting with RNase A, and purifying on a new QIAGEN-tip as detailed in "Purification of plasmid DNA prepared by other methods" on our Web page www.qiagen.com/goto/plasmidinfo.
c)	Nuclease contamination	Check buffers for nuclease contamination and replace if necessary. Use new glass- and plasticware, and wear gloves.
d)	Lysis time was too long	Ensure that the lysis step (Buffer P2) does not exceed 5 min.
e)	Overloaded alkaline lysis	Check the culture volume and yield against the capacity of the QIAGEN-tip. Reduce the culture volume accordingly or alternatively increase the volumes of Buffers P1, P2, and P3.
f)	Plasmid DNA is nicked/ sheared/degraded	DNA was poorly buffered. Redissolve DNA in Buffer TE, pH 8.0, to inhibit nuclease activity and maintain stable pH during storage.

		Comments and suggestions
g)	Endonuclease-containing host	Refer to background information on our Web page (www.qiagen.com/goto/plasmidinfo), and consider changing *E. coli* host strain.
h)	Shearing during redissolving	Redissolve DNA gently, without vortexing or vigorous pipetting. Avoid using small pipet tips.
i)	Particles in redissolved DNA	Centrifuge the DNA solution and transfer supernatant to a new tube. The particles have no affect on DNA quality. Alternatively, use HiSpeed Kits containing QIAprecipitator, which filters the eluate.

Poor DNA performance

a)	Too much salt in pellet	Ensure that isopropanol is at room temperature for precipitation, and wash the pellet twice with room temperature 70% ethanol. Precipitate the DNA again to remove the salt.
b)	Residual protein	Check culture volume against the recommended volumes and reduce if necessary. Ensure that the bacterial lysate is cleared properly by centrifugation at ≥20,000 x *g* for 45 min, or using a QIAfilter Cartridge.

Extra DNA bands on analytical gel

a)	Dimer form of plasmid	Dimers or multimers of supercoiled plasmid DNA are formed during replication of plasmid DNA. Typically, when purified plasmid DNA is electrophoresed, both the supercoiled monomer and dimer form of the plasmid are detected upon ethidium bromide staining of the gel (see Figure 3, page 35). The ratio of these forms is often host dependent.
b)	Plasmid has formed denatured supercoils	This species runs faster than closed circular DNA on a gel and is resistant to restriction digestion (see Figure 3, page 35). Do not incubate cells for longer than 5 min in Buffer P2. Mix immediately after addition of Buffer P3.
c)	Possible deletion mutants	Some sequences are poorly maintained in plasmids. Check for deletions by restriction analysis. Cosmid clones, in particular, should always be prepared from freshly streaked, well-isolated colonies, since cosmids are not stable in *E. coli* for long periods of time.

	Comments and suggestions
Blocked QIAGEN-tip	
Lysate was turbid	Ensure that the lysate is clear before it is loaded onto the column. Ensure that Buffer P3 is chilled before use. Check *g*-force and centrifugation time. Alternatively, clear the lysate using a QIAfilter Cartridge. To clear a blocked QIAGEN-tip, positive pressure may be applied (e.g., by using a syringe fitted into a rubber stopper with a hole).

QIAfilter Cartridges

		Comments and suggestions
QIAfilter Cartridge clogs during filtration		
a)	Too large culture volume used	Use no more than the culture volume recommended in the protocol.
b)	Inefficient mixing after addition of Buffer P3	Mix well until a fluffy white material has formed and the lysate is no longer viscous.
c)	Mixing too vigorous after addition of Buffer P3	After addition of Buffer P3 the lysate should be mixed immediately but gently. Vigorous mixing disrupts the precipitate into tiny particles which may clog the QIAfilter Cartridge.
d)	QIAfilter Cartridge was not loaded immediately after addition of Buffer P3	After addition of Buffer P3 the lysate should be poured immediately into the QIAfilter Cartridge. Decanting after incubation may disrupt the precipitate into tiny particles which may clog the QIAfilter Cartridge.
e)	QIAfilter Cartridge was agitated during incubation	Pour the lysate into the QIAfilter Cartridge immediately after addition of Buffer P3 and do not agitate during the 10 min incubation. Agitation causes the precipitate to be disrupted into tiny particles, instead of forming a layer.
f)	Incubation after addition of Buffer P3 on ice instead of at RT	Ensure incubation is performed at room temperature in the QIAfilter Cartridge. Precipitate flotation is more efficient at room temperature than on ice.

		Comments and suggestions
g)	Incubation time after addition of Buffer P3 too short	Incubate with Buffer P3 as indicated in the protocol. If the precipitate has not risen to the top after the 10 min incubation, carefully run a sterile pipet tip or sterile spatula around the cartridge wall to dislodge the precipitate before continuing with the filtration.
h)	Vacuum was weak or negligible (QIAfilter Mega-Giga Cartridges only)	Ensure that the vacuum source generates a vacuum pressure of –200 to –600 millibars (–150 to –450 mm Hg)

QIAfilter Cartridge clogs after addition of Buffer FWB2
(QIAfilter Mega-Giga Cartridges only)

Precipitate was not stirred after addition of Buffer FWB2	After addition of Buffer FWB2 to the QIAfilter Mega-Giga Cartridge, gently stir the precipitate using a sterile spatula.

Lysate not clear after filtration
(QIAfilter Midi and Maxi Cartridges only)

Precipitate was forced through the QIAfilter Cartridge	Filter until all of the lysate has passed through the QIAfilter Midi or Maxi Cartridge, but do not apply extreme force. Approximately 10 ml (QIAfilter Midi) or 25 ml (QIAfilter Maxi) of the lysate are typically recovered.

EndoFree Plasmid Kits

		Comments and suggestions
Endotoxin content higher than expected		
a)	Incubation time with Buffer ER too short	Ensure that the lysate is incubated on ice for 30 min for efficient endotoxin removal. Immediately after addition of Buffer ER the lysate appears turbid, but becomes clear again during ice incubation. The clearing of the lysate indicates sufficient incubation time.

b)	Recontamination of DNA after preparation	Use only plastic- and glassware that is certified to be pyrogen- or endotoxin-free. Never autoclave plastic- or glassware in autoclaves that have previously been used for bacteria. Use only water that is certified to be endotoxin-free for preparation of 70% ethanol. Resuspend the DNA in endotoxin-free Buffer TE.

Lysate becomes turbid during the binding step on the QIAGEN-tip

This is due to the temperature change from ice incubation to the binding step at room temperature, and has no negative effect on the performance of EndoFree Kits.

Appendix A: Agarose Gel Analysis of the Purification Procedure

DNA yields and quality can be readily analyzed by agarose gel electrophoresis. Poor yields and quality can be caused by a number of different factors. To determine the stage of the procedure where the problem occurred, save fractions from different steps of the purification procedure (see below and Table 3), and analyze by agarose gel electrophoresis.

Preparation of samples

Remove aliquots from the cleared lysate (sample 1), flow-through (sample 2), combined Buffer QC wash fractions (sample 3), and Buffer QN eluate (sample 4), as indicated in each protocol and in Table 3. Precipitate the nucleic acids with 1 volume of isopropanol, rinse the pellets with 70% ethanol, drain well, and resuspend in 10 µl Buffer TE, pH 8.0.

Table 5. Sample volumes required for agarose gel analysis

Sample	Protocol step	Maxi	Mega	Giga
1	Cleared lysate	120 µl	120 µl	75 µl
2	Flow-through	120 µl	120 µl	75 µl
3	Combined wash fraction	240 µl	160 µl	120 µl
4	Eluate	60 µl	22 µl	20 µl
(% of prep represented by each sample volume)		0.40%	0.08%	0.02%

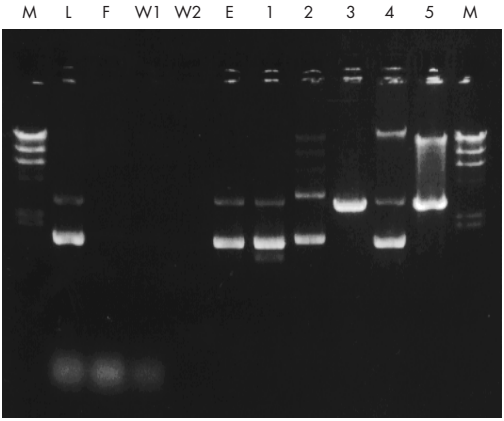


Figure 3. Agarose gel analysis of the plasmid purification procedure.

Agarose gel analysis

Run 2 µl of each sample on a 1% agarose gel* for analysis of the fractions at each stage of the plasmid purification procedure. Figure 3 shows an analytical gel of the different fractions, together with examples of problems that can arise at each step. If you find that you have a problem with a particular step of the protocol, turn to the hints in the relevant section of the troubleshooting guide starting on page 26. If the problem remains unresolved, or if you have any further questions, please call QIAGEN Technical Service.

L: Cleared lysate containing supercoiled and open circular plasmid DNA and degraded RNA (sample 1).

F: Flow-through fraction containing only degraded RNA is depleted of plasmid DNA which is bound to the QIAGEN resin (sample 2).

W1: First wash fraction, in which the remaining traces of RNA are removed without affecting the binding of the DNA (sample 3).

W2: Second wash fraction, which ensures that the resin is completely cleared of RNA and other contaminants, leaving only pure plasmid DNA on the column (sample 3).

E: The eluate containing pure plasmid DNA with no other contaminating nucleic acids (sample 4).

M: Lambda DNA digested with *Hin*dIII.

Lanes 1–5 illustrate some atypical results that may be observed in some preparations, depending on plasmid type and host strain.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

Lane 1: Supercoiled (lower band) and open circular form (upper band) of the high-copy plasmid pUC18 with an additional band of denatured supercoiled DNA migrating just below the supercoiled form. This form may result from prolonged alkaline lysis with Buffer P2 and is resistant to restriction digestion.

Lane 2: Multimeric forms of supercoiled plasmid DNA (pTZ19) which may be observed with some host strains, and should not be mistaken for genomic DNA. Multimeric plasmid DNA can easily be distinguished from genomic DNA by a simple restriction digestion — linearization of a plasmid sample displaying multimeric bands will yield a single defined band with the size of the linearized plasmid monomer (see lane 3).

Lane 3: Linearized form of plasmid pTZ19 after restriction digestion with *Eco*RI.

Lane 4: Sample contaminated with bacterial chromosomal DNA, which may be observed if the lysate is treated too vigorously (e.g., vortexing during incubation steps with Buffer P2 or P3). Genomic DNA contamination can easily be identified by digestion of the sample with *Eco*RI. A smear is observed, in contrast to the linear band seen after digestion of multimeric plasmid forms.

Lane 5: *Eco*RI digestion of a sample contaminated with bacterial genomic DNA which gives a smear above the plasmid DNA.

Appendix B: Removal of Bacterial Endotoxins

What are endotoxins?

Endotoxins, also known as lipopolysaccharides or LPS, are cell membrane components of Gram-negative bacteria (e.g., *E. coli*). The lipid portion of the outer layer of the outer membrane is completely composed of endotoxin molecules (Figure 4). A single *E. coli* cell contains about 2 million LPS molecules, each consisting of a hydrophobic lipid A moiety, a complex array of sugar residues and negatively charged phosphate groups (Figure 5). Therefore, each endotoxin molecule possesses hydrophobic, hydrophilic, and charged regions giving it unique features with respect to possible interactions with other molecules. Bacteria shed small amounts of endotoxins into their surroundings while they are actively growing and large amounts when they die. During lysis of bacterial cells for plasmid preparations, endotoxin molecules are released from the outer membrane into the lysate.

Endotoxin contamination of different plasmid preparation methods

The chemical structure and properties of endotoxin molecules and their tendency to form micellar structures lead to copurification of endotoxins with plasmid DNA. For example, in CsCl ultracentrifugation, the CsCl-banded DNA is easily contaminated with endotoxin molecules, which have a similar density in CsCl to plasmid–ethidium bromide complexes.

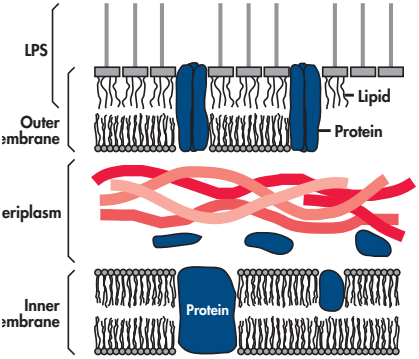


Figure 4. Schematic diagram of the envelope of *E. coli*.

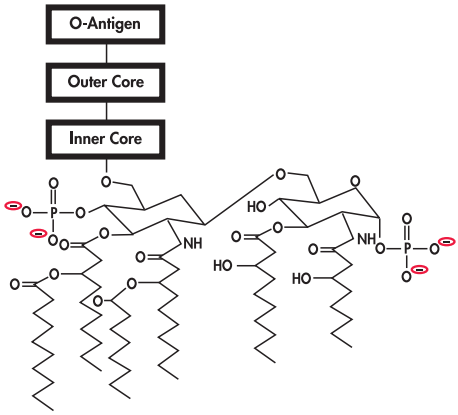


Figure 5. Schematic diagram of the endotoxin molecule.

On size exclusion resins, the large size of the micellar form of endotoxin causes the molecule to behave like a large DNA molecule; and in anion-exchange chromatography, the negative charges present on the endotoxin molecule can interact with anion-exchange resins, thus leading to copurification of endotoxins with the plasmid DNA.

However, the level of endotoxin contamination found in plasmid DNA is dependent on the purification method used. QIAGEN Plasmid Kits and 2 x CsCl gradient centrifugation both yield very pure DNA with relatively low levels of endotoxoin. Silica-slurry-purified DNA contains significantly higher endotoxin contamination. DNA purified with EndoFree Plasmid Kits contains only negligible amounts of endotoxin (<0.1 EU/µg plasmid DNA) (Table 4).

Table 6. Endotoxin contamination and transfection efficiency using various plasmid preparation methods*

Plasmid preparation method	Endotoxin (EU†/µg DNA)	Average transfection efficiency‡
EndoFree Plasmid Kits	0.1	154%
QIAGEN Plasmid Kits	9.3	100%
2x CsCl	2.6	99%
Silica slurry	1230.0	24%

* Host strain: DH5α; plasmid: pRSVcat.

† 1 ng LPS = 1.8 EU.

‡ The transfection efficiency obtained using plasmid prepared with QIAGEN Plasmid Kits was set to 100%. The transfection efficiencies for all other preparation methods were calculated relative to the QIAGEN Plasmid Kit.

How are endotoxins measured?

Historically, endotoxins were measured in a clotting reaction between the endotoxin and a clottable protein in the amoebocytes of *Limulus polyphemus*, the horseshoe crab. Today much more sensitive photometric tests (e.g., Kinetic-QCL Test from BioWhittaker, Inc.) are used, which are based on a *Limulus amoebocyte* lysate (LAL) and a synthetic color-producing substrate. LPS contamination is usually expressed in endotoxin units (EU). Typically, 1 ng LPS corresponds to 1–10 EU.

Influence of endotoxins on biological applications

Endotoxins strongly influence transfection of DNA into primary cells and sensitive cultured cells, and increased endotoxin levels lead to sharply reduced transfection efficiencies. Furthermore, it is extremely important to use endotoxin-free plasmid DNA for gene therapy applications, since endotoxins cause fever, endotoxic shock syndrome, and activation of the complement cascade in animals and humans. Endotoxins also interfere with in vitro transfection into immune cells such as macrophages and B cells by causing nonspecific activation of immune responses. These responses include the induced synthesis of immune mediators such as IL-1 and prostaglandin. It is important to make sure that plasticware, media, sera, and plasmid DNA are free of LPS contamination to avoid misinterpretation of experimental results.

Removal of endotoxins

The patented EndoFree Plasmid procedure (pages 14–25) integrates endotoxin removal into the standard QIAGEN Plasmid purification procedure. The neutralized bacterial lysate is filtered through a QIAfilter Cartridge and incubated on ice with a specific endotoxin removal buffer (Buffer ER). The endotoxin removal buffer prevents LPS molecules from binding to the resin in the QIAGEN-tips allowing purification of DNA containing less than 0.1 endotoxin units per µg plasmid DNA.

Endotoxin-free plasticware and glassware

To avoid recontamination of plasmid DNA after initial endotoxin removal, we recommend using only new plasticware which is certified to be pyrogen- or endotoxin-free. Endotoxin-free or pyrogen-free plasticware can be obtained from many different suppliers.

Endotoxins adhere strongly to glassware and are difficult to remove completely during washing. Standard laboratory autoclaving procedures have little or no effect on endotoxin levels. Moreover, if the autoclave has previously been used for bacteria, the glassware will become extensively contaminated with endotoxin molecules. Heating glassware at 180°C overnight is recommended to destroy any attached endotoxin molecules.

It is also important not to recontaminate the purified endotoxin-free DNA by using reagents that are not endotoxin-free. All buffers supplied with the EndoFree Plasmid Kits are tested and certified to be endotoxin-free, as are the water for preparation of 70% ethanol and the Buffer TE for resuspension.

Appendix C: Composition of Buffers

Buffer	Composition	Storage
Buffer P1 (resuspension buffer)	50 mM Tris·Cl, pH 8.0; 10 mM EDTA; 100 µg/ml RNase A	2–8°C, after addition of RNase A
Buffer P2 (lysis buffer)	200 mM NaOH, 1% SDS (w/v)	15–25°C
Buffer P3 (neutralization buffer)	3.0 M potassium acetate, pH 5.5	15–25°C or 2–8°C
Buffer FWB2 (QIAfilter wash buffer)	1 M potassium acetate pH 5.0	15–25°C
Buffer QBT (equilibration buffer)	750 mM NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol (v/v); 0.15% Triton® X-100 (v/v)	15–25°C
Buffer QC (wash buffer)	1.0 M NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol (v/v)	15–25°C
Buffer QF (elution buffer)	1.25 M NaCl; 50 mM Tris·Cl, pH 8.5; 15% isopropanol (v/v)	15–25°C
Buffer QN (elution buffer)	1.6 M NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol (v/v)	15–25°C
TE	10 mM Tris·Cl, pH 8.0; 1 mM EDTA	15–25°C
STE	100 mM NaCl; 10 mM Tris·Cl, pH 8.0; 1 mM EDTA	15–25°C

Preparation of buffers

Buffer compositions are given per liter of solution. Do not autoclave MOPS- or isopropanol-containing buffers; sterilize by filtration instead.

Buffer calculations are based on Tris base adjusted to pH with HCl (Tris·Cl). If using Tris·Cl reagent, the quantities used should be recalculated.

P1: Dissolve 6.06 g Tris base, 3.72 g $Na_2EDTA{\cdot}2H_2O$ in 800 ml distilled water. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 liter with distilled water. Add 100 mg RNase A per liter of P1.

P2: Dissolve 8.0 g NaOH pellets in 950 ml distilled water, 50 ml 20% SDS (w/v) solution. The final volume should be 1 liter.

P3: Dissolve 294.5 g potassium acetate in 500 ml distilled water. Adjust the pH to 5.5 with glacial acetic acid (~110 ml). Adjust the volume to 1 liter with distilled water.

FWB2: Dissolve 98.2 g potassium acetate in 500 ml distilled water. Adjust the pH to 5.0 with glacial acetic acid (~36 ml). Adjust the volume to 1 liter with distilled water.

QBT: Dissolve 43.83 g NaCl, 10.46 g MOPS (free acid) in 800 ml distilled water. Adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol and 15 ml 10% Triton X-100 solution (v/v). Adjust the volume to 1 liter with distilled water.

QC: Dissolve 58.44 g NaCl and 10.46 g MOPS (free acid) in 800 ml distilled water. Adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with distilled water.

QF: Dissolve 73.05 g NaCl and 6.06 g Tris base in 800 ml distilled water and adjust the pH to 8.5 with HCl. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with distilled water.

QN: Dissolve 93.50 g NaCl and 10.46 g MOPS (free acid) in 800 ml distilled water and adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with distilled water.

STE: Dissolve 5.84 g NaCl, 1.21 g Tris base, and 0.37 g $Na_2EDTA{\cdot}2H_2O$ in 800 ml distilled water. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 liter with distilled water.

Note: Always recheck pH of buffers after preparation.

Preparation of LB medium

Dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 800 ml distilled water. Adjust the pH to 7.0 with 1 N NaOH. Adjust the volume to 1 liter with distilled water. Sterilize by autoclaving.

Ordering Information

Product	Contents	Cat. no.
EndoFree Plasmid Maxi Kit (10)	10 QIAGEN-tip 500, Reagents, 10 QIAfilter Maxi Cartridges, Endotoxin-free Buffers	12362
EndoFree Plasmid Mega Kit (5)	5 QIAGEN-tip 2500, Reagents, 5 QIAfilter Mega-Giga Cartridges, Endotoxin-free Buffers	12381
EndoFree Plasmid Giga Kit (5)*	5 QIAGEN-tip 10000, Reagents, 5 QIAfilter Mega-Giga Cartridges, Endotoxin-free Buffers	12391
QIAGEN Plasmid Kits — for purification of transfection-grade plasmid or cosmid DNA		
QIAGEN Plasmid Midi Kit (25)*	25 QIAGEN-tip 100, Reagents, Buffers	12143
QIAGEN Plasmid Maxi Kit (10)*	10 QIAGEN-tip 500, Reagents, Buffers	12162
QIAGEN Plasmid Mega Kit (5)*	5 QIAGEN-tip 2500, Reagents, Buffers	12181
QIAGEN Plasmid Giga Kit (5)	5 QIAGEN-tip 10000, Reagents, Buffers	12191
QIAGEN Plasmid Kits — for the fastest and most convenient purification of transfection-grade plasmid DNA suitable for all applications		
QIAGEN Plasmid *Plus* Maxi Kit (25)	25 QIAGEN Plasmid *Plus* Maxi Columns, Extender Tubes, Reagents, Buffers, 25 QIAfilter Maxi Cartridges	12963
QIAGEN Plasmid *Plus* Midi Kit (25)	25 QIAGEN Plasmid *Plus* Midi Columns, Extender Tubes, Reagents, Buffers, 25 QIAfilter Midi Cartridges	12943
QIAGEN Plasmid *Plus* Giga Kit (5)	5 QIAGEN Plasmid *Plus* Mega Columns, Extender Tubes, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12991
QIAGEN Plasmid *Plus* Mega Kit (5)	5 QIAGEN Plasmid *Plus* Mega Columns, Extender Tubes, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12981

* Other kit sizes are available; see www.qiagen.com.

Ordering Information

Product	Contents	Cat. no.
QIAGEN Plasmid *Plus* 96 BioRobot Kit (4)	For 4 x 96 plasmid minipreps: TurboFilter 96 Plates and Plasmid *Plus* 96 Plates, Buffers, Reagents, Flat-Bottom Blocks, S-Blocks, and Elution Microtubes; for use with the BioRobot Universal System	960241
QIAfilter Plasmid Kits — for fast purification of transfection-grade plasmid or cosmid DNA		
QIAGEN Plasmid Mini Kit (25)*	25 QIAGEN-tip 20, Reagents, Buffers	12123
QIAfilter Plasmid Midi Kit (25)*	25 QIAGEN-tip 100, Reagents, Buffers, 25 QIAfilter Midi Cartridges	12243
QIAfilter Plasmid Maxi Kit (10)*	10 QIAGEN-tip 500, Reagents, Buffers, 10 QIAfilter Maxi Cartridges	12262
QIAfilter Plasmid Mega Kit (5)	5 QIAGEN-tip 2500, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12281
QIAfilter Plasmid Giga Kit (5)†	5 QIAGEN-tip 10000, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12291
HiSpeed Plasmid Kits — for ultrafast purification of transfection-grade plasmid or cosmid DNA		
HiSpeed Plasmid Midi Kit (25)	25 HiSpeed Midi Tips, 25 QIAfilter Midi Cartridges, 25 QIAprecipitator Midi Modules plus Syringes, Reagents, Buffers	12643
HiSpeed Plasmid Maxi Kit (10)*	10 HiSpeed Maxi Tips, 10 QIAfilter Maxi Cartriges, 10 QIAprecipitator Maxi Modules plus Syringes, Reagents, Buffers	12662

* Other kit sizes are available; see www.qiagen.com.

† For purification of low-copy plasmids and cosmids, EndoFree Plasmid Mega Kits are a better choice than EndoFree Plasmid Giga Kits, due to the large culture volumes required and the limited capacity of the QIAfilter Mega-Giga Cartridge.

Ordering Information

Product	Contents	Cat. no.
QIAprep® Spin Kit — for purification of molecular biology grade plasmid DNA		
QIAprep Spin Miniprep Kit (50)*	50 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)	27104
CompactPrep® Plasmid Kits† — for fast purification of molecular biology grade plasmid DNA		
CompactPrep Plasmid Midi Kit (25)	25 CompactPrep Midi Columns, Extender tubes, Reagents, Buffers, LyseBlue	12843
CompactPrep Plasmid Maxi Kit (25)	25 CompactPrep Maxi Columns, Extender tubes, Reagents, Buffers, LyseBlue	12863
QIAGEN Large-Construct Kit — for purification of BAC, PAC, and P1 DNA or up to 200 µg cosmid DNA, free of genomic DNA		
QIAGEN Large-Construct Kit (10)	10 QIAGEN-tip 500, Reagents, Buffers, ATP-Dependent Exonuclease‡	12462
Transfection products		
PolyFect Transfection Reagent (1 ml)	For 25–65 transfections in 60 mm dishes or 50–100 transfections in 6-well plates	301105
Effectene Transfection Reagent (1 ml)	For 40 transfections in 60 mm dishes or 160 transfections in 12-well plates	301425
SuperFect Transfection Reagent (1.2 ml)	For 40 transfections in 60 mm dishes or 160 transfections in 12-well plates	301305

* Other kit sizes are available; see www.qiagen.com.

† CompactPrep Kits require use of a vacuum device for operation (e.g., QIAvac 24 Plus, cat. no. 19413).

‡ ATP solution for exonuclease digestion is not provided.

Ordering Information

Product	Contents	Cat. no.
Accessories		
QIAvac 24 Plus	Vacuum manifold for processing 1–24 spin columns: QIAvac 24 Plus Vacuum manifold, Luer Plugs, Quick Couplings	19413
QIArack	1 rack for 12 x QIAGEN-tip 20, 8 x QIAGEN-tip 100, 6 x QIAGEN-tip 500 or HiSpeed Midi Tips, 4 x QIAGEN-tip 2500 or HiSpeed Maxi Tips, and 10 QIAfilter Midi or Maxi Cartridges	19015
RNase A (17,500 U)	2.5 ml (100 mg/ml; 7000 units/ml, solution)	19101
Plasmid Buffer Set	Buffers P1, P2, P3, QBT, QC, QF, RNase A; for 100 plasmid mini-, 25 midi-, or 10 maxipreps	19046
EndoFree Plasmid Buffer Set	Buffers P1, P2, P3, QBT, QC, QN, ER, TE, Endotoxin-free water, RNase A; for 10 plasmid mega- or 5 giga preps (endotoxin-free)	19048
Buffer P1	500 ml Resuspension Buffer (RNase A not included)	19051
Buffer P2	500 ml Lysis Buffer	19052
Buffer P3	500 ml Neutralization Buffer	19053

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Notes

Trademarks:

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