QIAGEN® Plasmid Purification Handbook

For

QIAGEN Plasmid Midi, Maxi, Mega, and Giga Kits

QIAfilter™ Plasmid Midi, Maxi, Mega, and Giga Kits

EndoFree™ Plasmid Maxi, Mega, and Giga Kits

July 1999

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What's NEW in the *QIAGEN Plasmid Purification Handbook?*

This handbook is a revised and expanded edition of the *QIAGEN® Plasmid Handbook, January 1997*. The changes are a result of ongoing research and development at QIAGEN and valuable feedback from customers. Routine users of the January 1997 version should take some time to review the contents of this new handbook.

Please note the following new features:

- QIAfilter™ Plasmid Mega and Giga Kit Protocol — QIAfilter Plasmid Kits have scaled up and are now available for streamlined purification of up to 10 mg of plasmid or cosmid DNA — see pages 25–29.
- EndoFree™ Plasmid Mega and Giga Kit Protocol — EndoFree Plasmid Kits have scaled up and are now available for purification of up to 10 mg of endotoxin-free ultrapure plasmid or cosmid DNA — see pages 35–39. For extensive background information on endotoxins — see pages 64–66.
- A detailed protocol for purifying very low-copy number plasmids/cosmids — see page 40.
- A handy table of the recommended cell culture and buffer volumes for high-, low-, and very low-copy plasmid purification using all sizes of QIAGEN-tips — see back of handbook.
- A thoroughly revised and simplified troubleshooting guide to help you get the most out of your QIAGEN Plasmid Purification Kit every time.

Important: Handbooks and protocols are often revised and improved. Be sure to always use the current handbook or protocol provided with the product.

Kit contents provided at back of handbook.

Contents

Storage Conditions

QIAGEN-tips and QIAfilter Cartridges should be stored dry and at room temperature. They can be stored for at least two years without showing any reduction in performance, capacity, or quality of separation.

QIAGEN, QIAfilter, and 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 six months. Other buffers and RNase A stock solution can be stored for two years at room temperature.

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Services Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding any aspect of QIAGEN, QIAfilter, or EndoFree Plasmid Kits, or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are also a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors listed on the last page.

Introduction

QIAGEN Plasmid Purification Kits will dramatically change the way you isolate nucleic acids. The rapid purification protocol, based on the remarkable selectivity of patented QIAGEN Resin, allows the isolation of ultrapure supercoiled plasmid DNA with high yields in just hours. No expensive equipment such as ultracentrifuges and HPLC or toxic reagents such as phenol and ethidium bromide are required.

Plasmid and cosmid DNA purified with QIAGEN-tips is ideally suited for use in demanding applications such as transfection, automated or manual sequencing, and enzymatic modifications. QIAGEN continually strives to streamline and further develop nucleic acid purification to offer a complete plasmid purification system which satisfies all your needs (see comparison of QIAGEN Plasmid Kits in Table 1). For transfection, QIAGEN also offers the advanced transfection reagents SuperFect™ and Effectene™. These reagents, combined with the high-quality plasmid DNA obtained from QIAGEN, QIAfilter, and EndoFree Plasmid Kits, provide the highest-efficiency, lowest-toxicity transfection available with a broad spectrum of cell types (for ordering information, see page 77).

Comparison of QIAGEN Plasmid Purification Kits

Table 1. Comparison of QIAGEN Plasmid Purification Kits

	QIAGEN anion-exchange tips	QIAfilter Cartridges	Endotoxin Removal Buffer
	Ultrapure plasmid DNA	Fast lysate clearing	Complete endotoxin removal
QIAGEN Plasmid Kits	**YES**	no	no
QIAfilter Plasmid Kits	**YES**	**YES**	no
EndoFree Plasmid Kits	**YES**	**YES**	**YES**

QIAGEN Plasmid Kits — unsurpassed quality for more than a decade

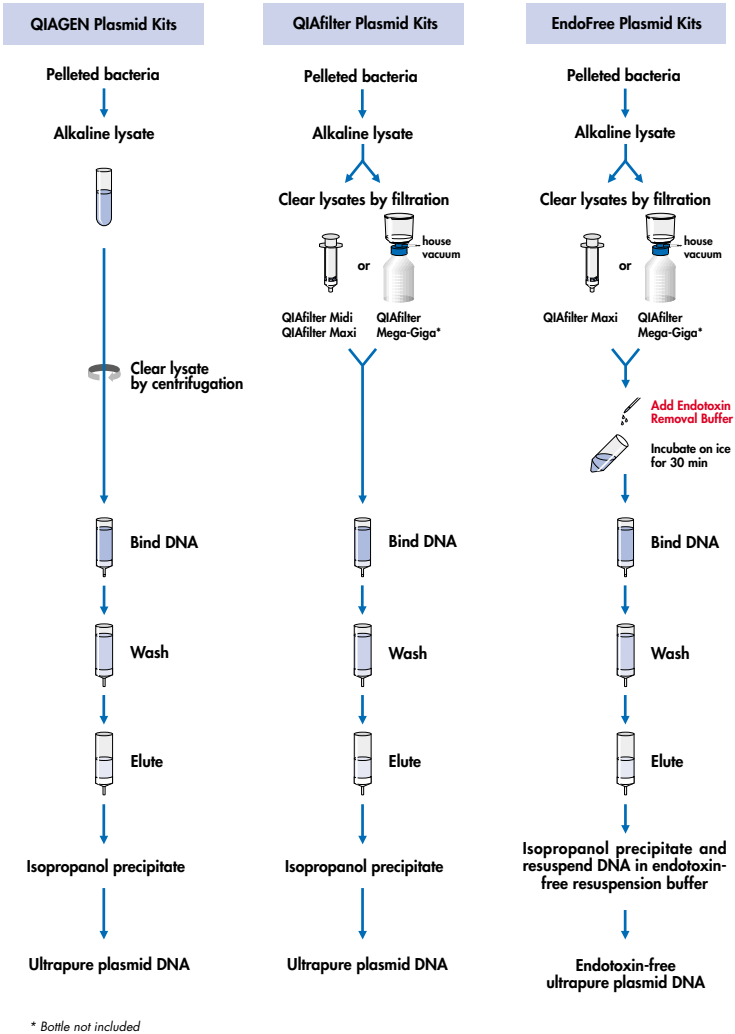
QIAGEN Plasmid Kits are designed for purification of up to 10 mg of ultrapure plasmid or cosmid DNA. QIAGEN Plasmid Kits use gravity-flow anion-exchange QIAGEN-tips for efficient purification of ultrapure DNA ideal for all applications from cloning to transfection.

QIAfilter Plasmid Kits — the faster alternative

QIAfilter Plasmid Kits are designed for streamlined purification of up to 10 mg of ultrapure plasmid or cosmid DNA. QIAfilter Plasmid Kits combine QIAfilter Cartridges for rapid clearing of bacterial lysates by filtration instead of centrifugation, with proven QIAGEN anion-exchange tips for streamlined DNA purification.

EndoFree Plasmid Kits — for endotoxin-free ultrapure DNA

EndoFree Plasmid Kits integrate an efficient endotoxin-removal step into the QIAGEN procedure for purification of up to 10 mg of endotoxin-free ultrapure plasmid DNA. Bacterial lysates are cleared by filtration with QIAfilter Cartridges, and plasmid DNA is purified on gravity-flow QIAGEN anion-exchange tips. Endotoxin-free DNA improves the efficiency of transfection into sensitive or immunologically active cells and is essential for gene therapy research.



** Bottle not included*

The QIAGEN Principle

QIAGEN plasmid purification protocols are based on a modified alkaline lysis procedure, followed by binding of plasmid DNA to QIAGEN Anion-Exchange 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. No expensive equipment such as ultracentrifuges and HPLC or toxic reagents such as phenol and ethidium bromide are required.

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 ideally suited for rapid and simple preparation of multiple samples.

QIAfilter Cartridges provided in QIAfilter and EndoFree Plasmid Kits enable rapid 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 3A). 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 3B). 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.

Brief Considerations for Plasmid/Cosmid Purification Procedures

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 pay particular attention to the "General Considerations for Optimal Results" section on pages 56–66, and be sure to follow the appropriate detailed protocol.

Plasmid size

Plasmids up to approximately 150 kb can be purified using QIAGEN plasmid purification protocols. Constructs larger than 45–50 kb, however, may exhibit somewhat reduced elution efficiencies. Prewarming the elution buffer to 50°C may help to increase the yield of large plasmids.

Plasmid/cosmid copy number

The protocols in this handbook are grouped according to the copy number of the plasmid or cosmid to be purified. High- and low-copy plasmids and cosmids (see page 57) should be purified using the standard protocols on pages 12–39. Very low-copy plasmids and very low-copy cosmids (<10 copies per cell) should be purified using the protocol on pages 40–44, which uses extremely large culture volumes to obtain good yields of very low-copy constructs.

Culture media

QIAGEN plasmid purification protocols are optimized for use with cultures grown in standard Luria Bertani 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 (see pages 59–61). 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 the QIAGEN-tips. If rich media must be used, growth time must be optimized, and culture volumes reduced (see pages 60–61).

Table 2. Composition of Luria Bertani medium

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

Please refer to Appendix A on page 68 for preparation of LB medium.

Culture volume

Do not exceed the maximum recommended culture volumes given at the beginning of each protocol. Using larger culture volumes can affect the efficiency of alkaline lysis, leading to reduced yield and purity of the preparation.

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 (Figure 1). The maximum plasmid binding capacities of the QIAGEN-tips 100, 500, 2500, and 10000 are 100 µg, 500 µg, 2.5 mg, and 10 mg, respectively. Actual yields will depend on culture volume, culture medium, plasmid copy number (see Table 5 on page 57), size of insert, and host strain.

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 2A). Alternatively, the QIAGEN-tips 100 and 500 may be placed in QIArack 2 (Figures 2B and 3A; cat. no. 19014) which has a removable collection tray.

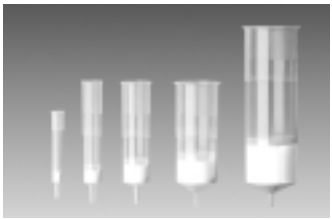


Figure 1. *QIAGEN-tip 20 to QIAGEN-tip 10000.*

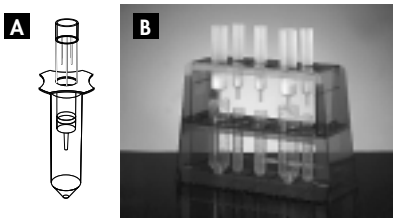


Figure 2. *Set-up of QIAGEN-tips* ***A*** *with tip holder or* ***B*** *with QIArack 2.*

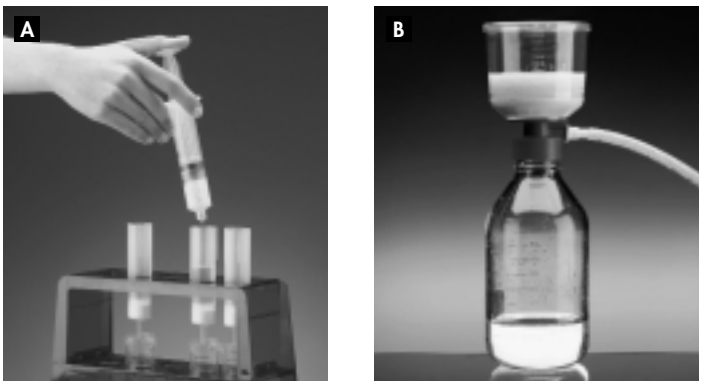


Figure 3. ***A*** *The syringe-format QIAfilter Maxi Cartridge in use with QIAGEN-tips in QIArack 2.*
B *The vacuum-operated QIAfilter Mega-Giga Cartridge in use. Note that the bottle is not included in kits.*

Analytical gel analysis

The success of the plasmid purification procedure can be monitored on an analytical gel (see Figure 4, page 46). 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 at what stage of the purification the problem occurred (see page 46).

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 may be stored at –20°C for several weeks. In addition, the DNA eluted from the QIAGEN-tip may 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.*

QIAGEN Plasmid Midi and Maxi Protocol

This protocol is designed for preparation of up to 100 µg of high- or low-copy plasmid or cosmid DNA using the QIAGEN Plasmid Midi Kit, or up to 500 µg using the QIAGEN Plasmid Maxi Kit. For purification of very low-copy plasmids or cosmids of less than 10 copies per cell, or double-stranded M13 replicative-form DNA, we recommend using the protocols on pages 40–45.

Low-copy plasmids that have been amplified in the presence of chloramphenicol should be treated as high-copy plasmids when choosing the appropriate culture volume.

Maximum recommended culture volumes

	QIAGEN-tip 100	QIAGEN-tip 500
High-copy plasmids	25 ml	100 ml
Low-copy plasmids	100 ml	500 ml

For the QIAGEN-tip 100, the expected yields are 75–100 µg for high-copy plasmids and 20–100 µg for low-copy plasmids. For the QIAGEN-tip 500, the expected yields are 300–500 µg for high-copy plasmids and 100–500 µg for low-copy plasmids.

Important notes before starting

- New users are strongly advised to read "General Considerations for Optimal Results" provided on pages 56–66 before starting the procedure.
- Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (spin down briefly before use) per bottle of Buffer P1, to give a final concentration of 100 µg/ml.
- 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.
- **Optional:** remove samples at the steps indicated with the symbol ☞ in order to monitor the procedure on an analytical gel (see page 46).

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 h at 37°C with vigorous shaking (~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 25 ml or 100 ml medium. For low-copy plasmids, inoculate 100 ml or 500 ml medium. Grow at 37°C for 12–16 h with vigorous shaking (~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 ml, which typically corresponds to a pellet wet weight of approximately 3 g/liter medium (see page 61).

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

   6000 x *g* corresponds to 6000 rpm in Sorvall GSA or GS3 or Beckman JA-10 rotors. Remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.

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

4. **Resuspend the bacterial pellet in 4 ml or 10 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. 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 or 10 ml of 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 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.

6. **Add 4 ml or 10 ml of chilled Buffer P3, mix immediately but gently by inverting 4–6 times, and incubate on ice for 15 min or 20 min.**

   Precipitation is enhanced by using chilled Buffer P3 and incubating on ice. 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 SDS. The lysate should be mixed thoroughly to avoid localized potassium dodecyl sulfate precipitation.

7. **Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Remove supernatant containing plasmid DNA promptly.**

   Before loading the centrifuge, the sample should be mixed again. Centrifugation should be performed in non-glass tubes (e.g., polypropylene). A centrifugal force of 20,000 x *g* corresponds to 12,000 rpm in a Beckman JA-17 rotor or 13,000 rpm in a Sorvall SS-34 rotor. After centrifugation the supernatant should be clear.

   Note: Instead of centrifugation steps 7 and 8, the lysate can be efficiently cleared by filtration using a QIAfilter Midi or Maxi Cartridge (see page 63).

8. **Re-centrifuge the supernatant at ≥20,000 x *g* for 15 min at 4°C. Remove supernatant containing plasmid DNA promptly.**

   This second centrifugation step should be carried out to avoid applying suspended or particulate material to the QIAGEN-tip. Suspended material (causing the sample to appear turbid) can clog the QIAGEN-tip and reduce or eliminate gravity flow.

   ☞ Remove a 240-µl or 120-µl sample from the cleared lysate supernatant and save for an analytical gel (sample 1) in order to determine whether growth and lysis conditions were optimal.

9. **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.**

   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.

10. **Apply the supernatant from step 8 to the QIAGEN-tip and allow it to enter the resin by gravity flow.**

    The supernatant should be loaded onto the QIAGEN-tip promptly. If it is left too long and becomes cloudy due to further precipitation of protein, it must be re-centrifuged or filtered before loading to prevent clogging of the QIAGEN-tip.

    ☞ Remove a 240-µl or 120-µl sample from the flow-through and save for an analytical gel (sample 2) in order to determine the efficiency of DNA binding to the QIAGEN Resin.

11. **Wash the QIAGEN-tip with 2 x 10 ml or 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 all contaminants in the majority of plasmid DNA preparations. The second wash is particularly necessary when large culture volumes or bacterial strains producing large amounts of carbohydrates are used.

    ☞ Remove a 400-µl or 240-µl sample from the combined wash fractions and save for an analytical gel (sample 3).

12. **Elute DNA with 5 ml or 15 ml Buffer QF.**

    Collect the eluate in a 10-ml or 30-ml tube. Use of polycarbonate centrifuge tubes is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

    ☞ Remove a 100-µl or 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.

13. **Precipitate DNA by adding 3.5 ml or 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 in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. A centrifugal force of 15,000 x *g* corresponds to 9,500 rpm in a Beckman JS-13 rotor and 11,000 rpm in a Sorvall SS-34 rotor. 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.

14. **Wash DNA pellet with 2 ml or 5 ml of room-temperature 70% ethanol, and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.**

    The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

15. **Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE, pH 8.0, or 10 mM Tris·Cl, pH 8.5).**

    Redissolve the DNA pellet by rinsing the walls to recover all 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 and quantitative analysis on an agarose gel.

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 at what stage of the purification procedure the problem occurred (see page 46).

QIAGEN Plasmid Mega and Giga Protocol

This protocol is designed for preparation of up to 2.5 mg of high- or low-copy plasmid or cosmid DNA using the QIAGEN Plasmid Mega Kit, or up to 10 mg using the QIAGEN Plasmid Giga Kit. For purification of very low-copy-number plasmids or cosmids of less than 10 copies per cell, or double-stranded M13 replicative form DNA, we recommend using the protocols on pages 40–45.

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.

Maximum recommended culture volumes*

	QIAGEN-tip 2500	QIAGEN-tip 10000
High-copy plasmids	500 ml (1.5 g pellet wet weight)†	2.5 liters (7.5 g pellet wet weight)†
Low-copy plasmids	2.5 liters (7.5 g pellet wet weight)†	5 liters† (15 g pellet wet weight)†‡

* *For the QIAGEN-tip 2500, the expected yields are 1.5–2.5 mg for high-copy plasmids and 0.5–2.5 mg for low-copy plasmids. For the QIAGEN-tip 10000, the expected yields are 7.5–10 mg for high-copy plasmids and 1–5 mg for low-copy plasmids.*

† *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 fermented cultures, please refer to the pellet wet weight instead of the recommended culture volumes.*

‡ *Requires doubled amounts of alkaline lysis buffers.*

Important notes before starting

- New users are strongly advised to read "General Considerations for Optimal Results" provided on pages 56–66 before starting the procedure.
- Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (spin down briefly before use) per bottle of Buffer P1, to give a final concentration of 100 µg/ml.
- 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.
- **Optional:** remove samples at the steps indicated with the symbol ☞ in order to monitor the procedure on an analytical gel.

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 ~8 h at 37°C with vigorous shaking (~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 500 ml or 2.5 liters medium. For low-copy plasmids, inoculate 2.5 liters or 5 liters medium. Grow at 37°C for 12–16 h with vigorous shaking (~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 ml, which typically corresponds to a pellet wet weight of approximately 3 g/liter medium (see pages 59–61).

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

   6000 x *g* corresponds to 6000 rpm in Sorvall GSA or GS3 or Beckman JA-10 rotors. Remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.

   Note: For Giga preparations of low-copy plasmids using 5 liters of culture, volumes of Buffers P1, P2, and P3 in steps 4–6 should be doubled, due to the very large number of cells harvested. For routine Giga preparation of low-copy plasmids, additional Buffers P1, P2, and P3 may need to be purchased (see page 78) or prepared (see page 68).

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

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

5. **Add 50 ml or 125 ml of 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 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.

6. **Add 50 ml or 125 ml of chilled Buffer P3, mix immediately but gently by inverting 4–6 times, and incubate on ice for 30 min. Mix the sample several times during the incubation on ice.**

   Precipitation is enhanced by using chilled Buffer P3 and incubating on ice. 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 SDS. The lysate should be mixed thoroughly to avoid localized potassium dodecyl sulfate precipitation.

7. **Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Remove supernatant containing plasmid DNA promptly.**

   Before loading the centrifuge, the sample should be mixed again. Centrifugation should be performed in 250-ml or 500-ml non-glass tubes (e.g., polypropylene). A centrifugal force of 20,000 x *g* corresponds to 11,500 rpm in a Beckman JA-14 rotor or 11,000 rpm in a Sorvall GSA rotor. After centrifugation the supernatant should be clear.

   Note: Instead of centrifugation steps 7 and 8, the lysate can be efficiently cleared by filtration using a QIAfilter Mega-Giga Cartridge (see page 63).

8. **Re-centrifuge the supernatant at ≥20,000 x *g* for 15 min at 4°C. Remove supernatant containing plasmid DNA promptly.**

   This step should be carried out to avoid applying suspended or particulate material to the QIAGEN-tip. Suspended material (causing the sample to appear turbid) can clog the QIAGEN-tip and reduce or eliminate gravity flow.

   ☞ Remove a 120-µl or 75-µl sample from the cleared lysate supernatant and save for an analytical gel (sample 1) in order to determine whether growth and lysis conditions were optimal.

9. **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.

10. **Apply the supernatant from step 8 to the QIAGEN-tip and allow it to enter the resin by gravity flow.**

    The supernatant should be loaded onto the QIAGEN-tip promptly. If it is left too long and becomes cloudy due to further precipitation of protein, it must be re-centrifuged or filtered before loading to prevent clogging of the QIAGEN-tip.

    ☞ Remove a 120-µl or 75-µl sample from the flow-through and save for an analytical gel (sample 2) in order to determine efficiency of DNA binding to the QIAGEN Resin.

11. **Wash the QIAGEN-tip with 2 x 100 ml or 2 x 300 ml Buffer QC.**

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

    ☞ Remove a 160-µl or 120-µl sample from the combined wash fractions and save for an analytical gel (sample 3).

12. **Elute DNA with 35 ml or 75 ml Buffer QF.**

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

    ☞ Remove a 22-µl or 15-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.

13. **Precipitate DNA by adding 24.5 ml or 52.5 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 in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. A centrifugal force of 15,000 x *g* corresponds to 9,500 rpm in a Beckman JS-13 rotor and 11,000 rpm in a Sorvall SS-34 rotor. 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.

14. **Wash DNA pellet with 7 ml or 10 ml of room-temperature 70% ethanol, and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.**

    The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

15. **Air-dry the pellet for 10–20 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE, pH 8.0, or 10 mM Tris·Cl, pH 8.5).**

    Redissolve the DNA pellet by rinsing the walls to recover all 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 and quantitative analysis on an agarose gel.

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 at what stage of the purification procedure the problem occurred (see page 46).

QIAfilter Plasmid Midi and Maxi Protocol

This protocol is designed for preparation of up to 100 µg of high- or low-copy plasmid or cosmid DNA using the QIAfilter Plasmid Midi Kit, or up to 500 µg using the QIAfilter Plasmid Maxi Kit. In this protocol, QIAfilter Cartridges are used instead of conventional centrifugation to clear bacterial lysates. For purification of double-stranded M13 replicative-form DNA, we recommend using the protocol on page 45.

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.

Maximum recommended culture volumes*

	QIAfilter Midi	QIAfilter Maxi
High-copy plasmids	25 ml	100 ml
Low-copy plasmids†	50 ml	250 ml

* *For high-copy plasmids, expected yields are 75–100 µg for the QIAfilter Plasmid Midi Kit and 300–500 µg for the QIAfilter Plasmid Maxi Kit. For low-copy plasmids, expected yields are 10–50 µg for the QIAfilter Plasmid Midi Kit and 50–250 µg for the QIAfilter Plasmid Maxi Kit using these culture volumes.*

† *The maximum recommended culture volumes apply to the capacity of the QIAfilter Midi and Maxi Cartridges. If higher yields of low-copy plasmids yields are desired, the lysates from two QIAfilter Midi Cartridges can be loaded onto one QIAGEN-tip 100, or the lysates from two QIAfilter Maxi Cartridges can be loaded onto one QIAGEN-tip 500.*

Important notes before starting

- New users are strongly advised to read "General Considerations for Optimal Results" provided on pages 56–66 before starting the procedure.
- Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (spin down briefly before use) per bottle of Buffer P1, to give a final concentration of 100 µg/ml.
- 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.
- In contrast to the standard protocol, the lysate is not incubated on ice after addition of Buffer P3.
- **Optional:** remove samples at the steps indicated with the symbol ☞ in order to monitor the procedure on an analytical gel.

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 h at 37°C with vigorous shaking (~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 25 ml or 100 ml medium. For low-copy plasmids, inoculate 50 ml or 250 ml medium. Grow at 37°C for 12–16 h with vigorous shaking (~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 ml, which typically corresponds to a pellet wet weight of approximately 3 g/liter medium (see pages 59–61).

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

   6000 x *g* corresponds to 6000 rpm in Sorvall GSA or GS3 or Beckman JA-10 rotors. Remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.

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

4. **Resuspend the bacterial pellet in 4 ml or 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. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

5. **Add 4 ml or 10 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 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.

   During the incubation prepare the QIAfilter Cartridge:

   Screw the cap onto the outlet nozzle of the QIAfilter Midi or QIAfilter Maxi Cartridge. Place the QIAfilter Cartridge in a convenient tube.

6. **Add 4 ml or 10 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.**

   Precipitation is enhanced by using chilled Buffer P3. After addition of Buffer P3, a fluffy white precipitate containing genomic DNA, proteins, cell debris, and SDS becomes visible. It is important to transfer the lysate into the QIAfilter Cartridge immediately in order to prevent later disruption of the precipitate layer.

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 or 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. **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.**

   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.

9. **Remove the cap from the QIAfilter outlet nozzle. Gently insert the plunger into the QIAfilter Midi or QIAfilter Maxi Cartridge and filter the cell lysate into the previously equilibrated QIAGEN-tip.**

   Filter until all of the lysate has passed through the QIAfilter Cartridge, but do not apply extreme force. Approximately 10 ml and 25 ml of the lysate are generally recovered after filtration.

   ☞ Remove a 240-µl or 120-µl sample of the filtered lysate and save for an analytical gel (sample 1) in order to determine whether growth and lysis conditions were optimal.

10. **Allow the cleared lysate to enter the resin by gravity flow.**

    ☞ Remove a 240-µl or 120-µl sample of the flow-through and save for an analytical gel (sample 2) in order to the efficiency of DNA binding to the QIAGEN Resin.

11. **Wash the QIAGEN-tip with 2 x 10 ml or 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 all contaminants in the majority of plasmid preparations. The second wash is particularly necessary when large culture volumes or bacterial strains producing large amounts of carbohydrates are used.

    ☞ Remove a 400-µl or 240-µl sample of the combined wash fractions and save for an analytical gel (sample 3).

12. **Elute DNA with 5 ml or 15 ml Buffer QF.**

    Collect the eluate in a 10-ml or 30-ml tube. Use of polycarbonate centrifuge tubes for collection is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

☞ Remove a 100-µl or 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.

13. **Precipitate DNA by adding 3.5 ml or 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 in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. A centrifugal force of 15,000 x *g* corresponds to 9,500 rpm in a Beckman JS-13 rotor and 11,000 rpm in a Sorvall SS-34 rotor. 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.

14. **Wash DNA pellet with 2 ml or 5 ml of room-temperature 70% ethanol and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.**

    The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

15. **Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE, pH 8.0, or 10 mM Tris·Cl, pH 8.5).**

    Redissolve the DNA pellet by rinsing the walls to recover all 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 and quantitative analysis on an agarose gel.

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 at what stage of the purification procedure the problem occurred (see page 46).

QIAfilter Plasmid Mega and Giga Protocol

This protocol is designed for preparation of up to 2.5 mg of high- or low-copy plasmid and cosmid DNA using the QIAfilter Plasmid Mega Kit, or up to 10 mg of high-copy plasmid DNA using the QIAfilter Plasmid Giga Kit. In this protocol QIAfilter Cartridges are used instead of conventional centrifugation to clear bacterial lysates. (Please note: The QIAfilter Plasmid Giga Kit is not recommended for low-copy plasmids or cosmids). For purification of double-stranded M13 replicative-form DNA, we recommend using the protocol on page 45.

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.

Maximum recommended culture volumes*

	QIAfilter Mega	QIAfilter Giga
High-copy number plasmids	500 ml LB culture (1.5 g pellet wet weight)†	2.5 liters LB culture (7.5 g pellet wet weight)†
Low-copy number 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 QIAfilter Plasmid Mega Kit and 7.5–10 mg for the QIAfilter Plasmid Giga Kit. For low-copy plasmids, expected yields are 0.5–2.5 mg for the QIAfilter Plasmid Mega Kit. The QIAfilter 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 QIAfilter Plasmid Mega Kit is a better choice than the QIAfilter Plasmid Giga Kit for purification of low-copy plasmids and cosmids.*

Important notes before starting

- New users are strongly advised to read "General Considerations for Optimal Results" on pages 56–66 before starting the procedure.
- 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.**
- Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (spin briefly before use) per bottle of Buffer P1 to give a final concentration of 100 µg/ml.
- 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.
- In contrast to the standard protocol, the lysate is not incubated on ice after addition of Buffer P3.
- **Optional:** remove samples at the steps indicated with the symbol ☞ in order to monitor the procedure on an analytical gel.

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 antibiotic. Incubate for ~8 h at 37°C with vigorous shaking (~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. For low-copy plasmids, inoculate 2.5 liters medium. Grow at 37°C for 12–16 h with vigorous shaking (~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 3–4 x 10^9 cells per ml, 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.**

   6000 x *g* corresponds to 6000 rpm in Sorvall GSA or GS3 or Beckman JA-10 rotors. Remove all traces of supernatant by inverting the open centrifuge bottle until all medium has been drained.

   ⊗ 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 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. 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 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 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.

7. **Add 50 ml or 125 ml chilled Buffer P3, and mix immediately and thoroughly by 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 SDS becomes visible. The lysate should be mixed well to reduce the viscosity and prevent clogging of the QIAfilter Cartridge.

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 FWB 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. The filtered lysate in the bottle contains the plasmid DNA.

    ☞ 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. 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.

12. Apply the filtered lysate from step 10 onto the QIAGEN-tip and allow it to enter the resin by gravity flow.

☞ Remove a 120-µl or 75-µl sample of the flow-through and save for an analytical gel (sample 2) in order to determine the efficiency of DNA binding to the QIAGEN Resin.

13. Wash the QIAGEN-tip with 2 x 100 ml or 2 x 300 ml Buffer QC.

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

☞ Remove a 160-µl or 120-µl sample of the eluate and save for an analytical gel (sample 3).

14. Elute DNA with 35 ml or 75 ml Buffer QF.

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

☞ Remove a 22-µl or 15-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.

15. Precipitate DNA by adding 24.5 ml or 52.5 ml room-temperature isopropanol (0.7 volumes) 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 in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. A centrifugal force of 15,000 x *g* corresponds to 9,500 rpm in a Beckman JS-13 rotor and 11,000 rpm in a Sorvall SS-34 rotor. Isopropanol DNA 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 easily located. Isopropanol DNA pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant.

16. **Wash DNA pellet with 7 ml or 10 ml of room-temperature 70% ethanol, and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.**

    The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

17. **Air-dry the pellet for approximately 10–20 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE, pH 8.0 or 10 mM Tris·Cl, pH 8.5).**

    Redissolve the DNA pellet by rinsing the walls to recover all 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 dissolve easily in acidic buffers.

Determination of yield

To determine the yield, DNA concentration should be determined by both UV spectrophotometry and quantitative analysis on an agarose gel.

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 at what stage of the purification procedure the problem occurred (see page 46).

EndoFree Plasmid Maxi Protocol

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 64–66.

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.

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 notes before starting

- New users are strongly advised to read "General Considerations for Optimal Results" provided on pages 56–66 before starting the procedure.
- Use endotoxin-free plastic pipet tips and tubes for elution and subsequent steps (step 13 onwards). Endotoxin-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.
- Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (spin down briefly before use) per bottle of 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.
- In contrast to the standard protocol there is no incubation on ice after addition of Buffer P3.
- **Optional:** remove samples at the steps indicated with the symbol ☞ in order to monitor the procedure on an analytical gel.

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 h at 37°C with vigorous shaking (~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, and for low-copy plasmids, inoculate 250 ml medium. Grow at 37°C for 12–16 h with vigorous shaking (~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 ml, which typically corresponds to a pellet wet weight of approximately 3 g/liter medium (see page 59–61).

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

   6000 x *g* corresponds to 6000 rpm in Sorvall GSA or GS3 or Beckman JA-10 rotors. Remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.

   ⊗ 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. 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 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 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.

   During the incubation prepare the QIAfilter Cartridge:

   Screw the cap onto the outlet nozzle of the QIAfilter Maxi Cartridge. Place the QIAfilter Cartridge into a convenient tube.

6. **Add 10 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.**

   Precipitation is enhanced by using chilled Buffer P3. After addition of Buffer P3, a fluffy white precipitate containing genomic DNA, proteins, cell debris, and SDS becomes visible. It is important to transfer the lysate into the QIAfilter Cartridge immediately in order to prevent later disruption of the precipitate layer.

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 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.

   ☞ Remove a 120-µl sample of the filtered lysate and save for an analytical gel (sample 1) in order 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.

    ☞ Remove a 120-µl sample of the flow-through and save for an analytical gel (sample 2) in order 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 all 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.

☞ 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 pre-treated glassware.

13. **Elute DNA with 15 ml Buffer QN.**

    Collect the eluate in a 30-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.

    ☞ 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 in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. A centrifugal force of 15,000 x *g* corresponds to 9,500 rpm in a Beckman JS-13 rotor and 11,000 rpm in a Sorvall SS-34 rotor. 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.**

    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 all 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 and quantitative analysis on an agarose gel.

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 at what stage of the purification procedure the problem occurred (see page 46).

EndoFree Plasmid Mega and Giga Protocol

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 64–66. (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.

Maximum recommended culture volumes*

	EndoFree Mega	EndoFree Giga
High-copy number plasmids	500 ml LB culture (1.5 g pellet wet weight)†	2.5 liters LB culture (7.5 g pellet wet weight)†
Low-copy number 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 notes before starting

- New users are strongly advised to read the "General Considerations for Optimal Results" section on pages 56–66 before starting the procedure.
- Use endotoxin-free plastic pipet tips and tubes for elution and subsequent steps (step 15 onwards). Endotoxin-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.
- Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (spin briefly before use) per bottle of 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 H_2O supplied with the kit.
- Check Buffer P2 for SDS precipitation due to low storage temperatures and, if necessary, dissolve the SDS by warming to 37°C.
- Pre-chill Buffer P3 to 4°C.
- In contrast to the standard protocol, the lysate is not incubated on ice after addition of Buffer P3.
- **Optional:** remove samples at the steps indicated with the symbol ☞ in order to monitor the procedure on an analytical gel.

Procedure

1. **Pick a single colony from a selective plate and inoculate a starter culture of 5–10 ml LB medium containing the appropriate selective agent. Grow for ~8 h at 37°C with vigorous shaking (~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. For low-copy plasmids, inoculate 2.5 liters medium. Grow at 37°C for 12–16 h with vigorous shaking (~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 3–4 x 10^9 cells per ml, which typically corresponds to a pellet wet weight of approximately 3 g/liter medium (see pages 59–61).

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

   6000 x *g* corresponds to 6000 rpm in Sorvall GSA or GS3 or Beckman® JA-10 rotors. Remove all traces of supernatant by inverting the open centrifuge bottle until all medium has been drained.

   ⊗ 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 Buffer P1.**

   Note: 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. 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 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 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.

7. **Add 50 ml or 125 ml chilled Buffer P3, and mix immediately and thoroughly by inverting 4–6 times. Mix well until a 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 SDS becomes visible. The lysate should be mixed well to reduce the viscosity and prevent clogging of the QIAfilter Cartridge.

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 (Mega and Giga) Buffer FWB 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. The filtered lysate in the bottle contains the plasmid DNA.

    ☞ 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.

    ☞ Remove a 120-µl or 75-µl sample of the flow-through and save for an analytical gel (sample 2) in order to determine the efficiency of DNA binding to the QIAGEN Resin.

14. **Wash the QIAGEN-tip with 2 x 100 ml or 2 x 300 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 enough to remove all 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.

    ☞ Remove a 160-µl or 120-µl sample of the eluate and save for an analytical gel (sample 3).

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

15. **Elute DNA with 35 ml or 75 ml Buffer QN.**

    Drain the QIAGEN-tip by allowing it to empty by gravity flow. Use of polycarbonate centrifuge tubes for collection is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

    ☞ Remove a 22-µl or 15-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.

16. **Precipitate DNA by adding 24.5 ml or 52.5 ml room-temperature isopropanol (0.7 volumes) 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 in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. A centrifugal force of 15,000 x *g* corresponds to 9,500 rpm in a Beckman JS-13 rotor and 11,000 rpm in a Sorvall SS-34 rotor. Isopropanol DNA 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 easily located. Isopropanol DNA 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 H_2O supplied with the kit) and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.**

    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 approximately 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 all 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 dissolve easily in acidic buffers.

Determination of yield

To determine the yield, DNA concentration should be determined by both UV spectrophotometry and quantitative analysis on an agarose gel.

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 at what stage of the purification procedure the problem occurred (see page 46).

Very Low-Copy Plasmid/Very Low-Copy Cosmid Purification Protocol

Very low-copy plasmids and cosmids of less than 10 copies per cell (see page 57) often require large culture volumes to yield significant amounts of DNA. This protocol is suitable for QIAGEN-tip 100 or QIAGEN-tip 500, and uses centrifugation to clear lysates rather than QIAfilter Cartridges, due to the large culture volumes. After alkaline lysis, there is an additional isopropanol precipitation step to decrease the amount of lysate before DNA is bound to the QIAGEN-tip. Culture volumes and tip sizes are selected to match the quantity of DNA expected to the capacity of the QIAGEN-tip. For purification of P1 and BAC DNA using QIAGEN-tips, please contact one of our technical service groups or your local distributor (see page 79).

Details of yields, culture volumes, QIAGEN-tip sizes, and buffer volumes to be used for purification of very low-copy plasmids and cosmids are given in Table 3.

Table 3. Parameters for purification of very low-copy plasmids and cosmids of less than 10 copies per cell

Required DNA yield*	**Up to 100 µg**	**Up to 500 µg**
Culture volume	500 ml	2.5 liters
Buffer P1†	20 ml	125 ml
Buffer P2†	20 ml	125 ml
Buffer P3†	20 ml	125 ml
QIAGEN-tip	**QIAGEN-tip 100**	**QIAGEN-tip 500**
Buffer QBT (for equilibration)	4 ml	10 ml
Buffer QC (for washing)	2 x 10 ml	2 x 30 ml
Buffer QF (for elution)	5 ml	15 ml

* *For very low-copy plasmids, expected yields are 20–100 µg for the QIAGEN-tip 100 and 100–500 µg for the QIAGEN-tip 500.*

† *Volumes of lysis Buffers P1, P2, and P3 are higher than in the standard protocols on pages 12–39 in order to efficiently lyse the large number of cells required for purification of very low-copy plasmids and cosmids.*

Important notes before starting

- New users are strongly advised to read "General Considerations for Optimal Results" provided on pages 56–66 before starting the procedure.
- Add the provided RNase A solution to Buffer P1 before use. Use one vial of RNase A (spin down briefly before use) per bottle of Buffer P1, to give a final concentration of 100 µg/ml.
- 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.
- **Optional:** remove samples at the steps indicated with the symbol ☞ in order to monitor the procedure on an analytical gel.

Procedure

1. **Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–10 ml LB medium containing the appropriate selective antibiotic. Incubate for ~8 h at 37°C with vigorous shaking (~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 500 ml or 2.5 liters of selective LB medium. Grow at 37°C for 12–16 h with vigorous shaking (~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 ml , which typically corresponds to a pellet wet weight of approximately 3 g/liter medium (see pages 59–61).

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

   6000 x *g* corresponds to 6000 rpm in Sorvall GSA or GS3 or Beckman JA-10 rotors. Remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.

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

4. **Resuspend the bacterial pellet in 20 ml or 125 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 the 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 20 ml or 125 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 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.

6. **Add 20 ml or 125 ml chilled Buffer P3, mix immediately but gently by inverting 4–6 times, and incubate on ice for 30 min. Mix sample several times during the incubation on ice.**

   Precipitation is enhanced by using chilled Buffer P3 and incubating on ice. 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 SDS. The lysate should be mixed thoroughly to avoid localized potassium dodecyl sulfate precipitation.

7. **Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Remove supernatant containing plasmid DNA promptly.**

   Before loading the centrifuge, the sample should be mixed again. Centrifugation should be performed in non-glass tubes (e.g., polypropylene). A centrifugal force of 20,000 x *g* corresponds to 12,000 rpm in a Beckman JA-17 rotor or 11,500 rpm in a Beckman JA-14 rotor. After centrifugation, the supernatant should be clear.

8. **Re-centrifuge the supernatant at ≥20,000 x *g* for 15 min at 4°C. Remove supernatant containing plasmid DNA promptly. Alternatively, the sample can be filtered over a prewetted, folded filter.**

   This second centrifugation step clears the lysate completely of precipitated material.

   ☞ Remove a 600-µl or 750-µl sample from the cleared lysate supernatant and save for an analytical gel (sample 1) to determine whether growth and lysis conditions were optimal.

9. **Precipitate the DNA by adding 42 ml or 262.5 ml (0.7 volumes) of room-temperature isopropanol to the lysate. Centrifuge at ≥15,000 x *g* for 30 min at 4°C, and carefully decant the supernatant.**

   This isopropanol precipitation reduces the sample volume to facilitate loading of the column. It also serves to remove unwanted metabolites such as proteins and lipopolysaccharides. A centrifugal force of 15,000 x *g* corresponds to 10,000 rpm in a Beckman JA-14 rotor or 9,500 rpm in a Beckman JA-10 rotor.

10. **Redissolve the DNA pellet in 500 µl TE, pH 7.0, and add Buffer QBT to obtain a final volume of 5 ml or 12 ml for selected QIAGEN-tip 100 or QIAGEN-tip 500, respectively.**

    TE buffer is used to facilitate redissolving of the DNA. Buffer QBT provides optimal DNA binding conditions.

11. **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.**

    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.

12. **Apply the DNA solution from step 10 to the QIAGEN-tip and allow it to enter the resin by gravity flow.**

    ☞ Remove a 50-µl or 24-µl sample from the flow-through and save for an analytical gel (sample 2) in order to determine the efficiency of DNA binding to the QIAGEN Resin.

13. **Wash the QIAGEN-tip with 2 x 10 ml or 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 all contaminants in the majority of plasmid DNA preparations. The second wash is particularly necessary when large culture volumes or bacterial strains producing large amounts of carbohydrates are used.

    ☞ Remove a 200-µl or 120-µl sample from the combined wash fractions and save for an analytical gel (sample 3).

14. **Elute DNA with 5 ml or 15 ml Buffer QF.**

    Use of polycarbonate tubes to collect the eluate is not recommended as polycarbonate is not resistant the alcohol used in subsequent steps.

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

    ☞ Remove a 50-µl or 30-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.

15. **Precipitate DNA by adding 3.5 ml or 10.5 ml (0.7 volumes) of 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 in order to minimize salt precipitation, although centrifugation is carried out at 4°C to prevent overheating of the sample. A centrifugal force of 15,000 x *g* corresponds to 9,500 rpm in a Beckman JS-13 rotor and 11,000 rpm in a Sorvall SS-34 rotor. 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.

16. **Wash DNA pellet with 2 ml or 5 ml room-temperature 70% ethanol, and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.**

    The 70% ethanol removes precipitated salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

17. **Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE, pH 8.0, or 10 mM Tris·Cl, pH 8.5).**

    Redissolve DNA pellet by rinsing the walls to recover all 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 and quantitative analysis on an agarose gel.

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 at what stage of the purification procedure the problem occurred (see page 46).

Special Protocols for:

Purification of plasmid DNA prepared by other methods*

Before using this protocol, the DNA must be free of SDS and other anionic detergents. If the prep is contaminated with RNA, the RNA must first be digested with RNase A (cat. no. 19101). Note that QIAGEN-tips cannot separate plasmid DNA from chromosomal DNA — this separation is achieved during the alkaline lysis procedure.

1. Choose a QIAGEN-tip appropriate for the amount of DNA to be purified. QIAGEN-tips 100, 500, 2500, and 10000 are appropriate for purifying up to 100 µg, 500 µg, 2.5 mg, and 10 mg DNA, respectively.
2. Either adjust the DNA sample to 750 mM NaCl, 50 mM MOPS, pH 7.0, or resuspend the DNA sample in Buffer QBT. (The volume of Buffer QBT added should be at least 10 times the volume of the original sample solvent.) The final sample volume should be at least 5, 12, 40, or 90 ml for QIAGEN-tips 100, 500, 2500, or 10000, respectively.
3. Apply the sample to a QIAGEN-tip previously equilibrated with Buffer QBT.
4. Proceed with step 11 in the detailed QIAGEN Plasmid Protocol (see page 14 for Midi/Maxi preparations and page 19 for Mega/Giga preparations).

Purification of M13 replicative form*

M13 RF DNA can be purified using any of the QIAGEN or QIAfilter Plasmid Protocols on pages 12–29. For choice of culture volume, M13 RF DNA should be treated as a low-copy-number plasmid. The only modification to the purification procedures is an extra wash step before cell lysis in order to remove all traces of phage supernatant.

1. Resuspend bacterial pellet in 10 ml, 50 ml, 250 ml, or 500 ml STE buffer (see pages 67 and 68 for composition and preparation, respectively) for Midi, Maxi, Mega, or Giga, preparations respectively. Pellet cells again and carefully remove all the supernatant.
2. Continue with step 4 of the appropriate plasmid protocol (see page 13 for Midi/Maxi preparations, page 17 for Mega/Giga preparations, page 22 for Midi/Maxi preparations using QIAfilter, or page 26 for Mega/Giga preparations using QIAfilter).

** For further information, please contact our Technical Services Department or your local distributor listed on the last page.*

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 at what stage of the procedure any problem occurred, save fractions from different steps of the purification procedure (see below and Table 4), 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 QF/QN eluate (sample 4), as indicated in each protocol and in Table 4. Precipitate the nucleic acids with 1 volume of isopropanol, rinse the pellets with 70% ethanol, drain well, and resuspend in 10 µl TE, pH 8.0.

Table 4. Sample volumes required for agarose gel analysis

						Very low-copy plasmids/cosmids	
						QIAGEN-tip	
Sample	**Protocol step**	**Midi**	**Maxi**	**Mega**	**Giga**	**100**	**500**
1	Cleared lysate	240 µl	120 µl	120 µl	75 µl	600 µl	750 µl
2	Flow-through	240 µl	120 µl	120 µl	75 µl	50 µl	24 µl
3	Combined wash fractions	400 µl	240 µl	160 µl	120 µl	200 µl	120 µl
4	Eluate	100 µl	60 µl	22 µl	15 µl	50 µl	30 µl
(% of prep represented by each sample volume)		2.00%	0.40%	0.08%	0.02%	1.00%	0.20%

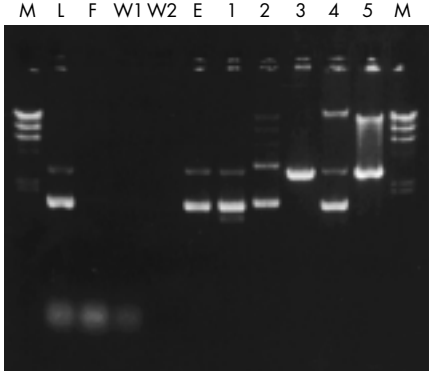


Figure 4. *Agarose gel analysis of the plasmid purification procedure.*

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 4 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 on pages 49–56. If the problem remains unresolved, or if you have any further questions, please call QIAGEN Technical Services.

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.

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.

Reliability of DNA Quantitation by Spectrophotometry

The concentration of nucleic acids in solution can be readily calculated from the absorbance at 260 nm. Figure 5 shows the standard deviation of A_{260} measurements for serial dilutions of DNA. Although A_{260} values between 0.1 and 1.0 are very reproducible, A_{260} readings <0.1 and >1.0 lead to considerably lower reproducibility. Furthermore, readings above 3.0 are incorrect, which can potentially leading to underestimation of the DNA quantity. Therefore, for reliable spectrophotometric DNA quantitation, A_{260} readings should lie between 0.1 and 1.0. When working with small amounts of DNA, quantitation by agarose gel electrophoresis may be more reliable.

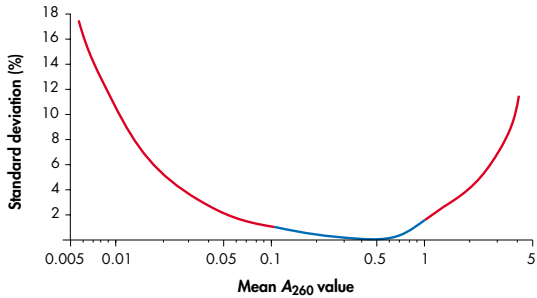


Figure 5. *Reliability of A_{260} readings. A_{260} readings were taken for serial dilutions of plasmid DNA. For each sample the percentage standard deviation was plotted against the mean A_{260} value from 4 measurements. The blue region of the curve represents the range of A_{260} readings which are most reproducible (0.1–1.0). The red regions represent A_{260} readings with high standard deviations.*

Troubleshooting Guide

Poor yields and quality can be caused by a number of different factors. For optimal plasmid-preparation conditions, particular attention should be paid to the lysis conditions as described in the protocol. In addition, adhering to our recommendations with respect to plasmid copy number, capacity of QIAGEN-tip, culture volume, and culture media, will ensure consistent and optimal results.

The following troubleshooting guide as well as "General Considerations for Optimal Results" provided on pages 56–66 of this manual may be helpful in solving any problems that may arise. The scientists at QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol(s) in this handbook or molecular biology applications (see page 79).

This troubleshooting guide is divided into three sections: the first details general troubleshooting relevant to all plasmid kits described in this handbook, the second is specifically for QIAfilter Cartridges, and the third for EndoFree Plasmid Kits.

QIAGEN, QIAfilter, and EndoFree Plasmid Purification Kits

	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" (pages 56–62), 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.
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 40 and Figure 7, page 61).

	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 P2 should always be closed immediately after use. Lysis buffers prepared in the laboratory should be prepared according to the instructions on page 68. 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 pages 40 and 60.
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.
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 68.
d) Column flow was uneven	Store QIAGEN-tips at room temperature. 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 larger a QIAGEN-tip if a higher yield is desired. For very low-copy number plasmid

	Comments and Suggestions
	and cosmid preps requiring very large culture volumes, please see pages 40 and 60.
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 on page 45.
No DNA in eluate (sample 4)	
a) No DNA in the lysate.	See page 49.
b) Elution Buffer QF or QN was incorrect	Check pH and salt concentration of Elution 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 longer at higher speeds. Try another isopropanol batch.
b) DNA pellet was lost	Isopropanol pellets are glassy and may be difficult to see. Mark the tube at the expected location of the pellet 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.

	Comments and Suggestions
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.
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 must be handled gently 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. 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 on page 45.
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 lysis step (Buffer P2) does not exceed 5 min.

	Comments and Suggestions
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 TE, pH 8.0, to inhibit nuclease activity and maintain stable pH during storage.
g) Endonuclease-containing host	Refer to page 58 of this handbook, and consider changing *E. coli* host strain.
h) Shearing during redissolving	Redissolve DNA gently, without vortexing or vigorous pipetting. Avoid using small pipet tips.
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. Re-precipitate the DNA 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 4, page 46). 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 4, page 46). Do not incubate cells for longer than 5 min in Buffer P2. Mix immediately after addition of Buffer P3.

	Comments and Suggestions
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.

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.

	Comments and Suggestions
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 at RT	Ensure incubation is performed at room temperature in the QIAfilter Cartridge. Precipitate flotation is more efficient at room temperature than on ice.
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 Cartridge 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 FWB
(QIAfilter Mega-Giga Cartridge only)

a) Precipitate was not stirred after addition of Buffer FWB	After addition of Buffer FWB 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)

a) 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) and 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 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.

General Considerations for Optimal Results

The QIAGEN plasmid purification procedure is an optimized protocol based on the alkaline lysis method of Birnboim and Doly (2). The procedure has been condensed to three steps and in combination with purification on QIAGEN Resin, allows selective preparation of ultrapure plasmid DNA without the use of phenol, chloroform, ethidium bromide, or cesium chloride. It can be used for the preparation of plasmid, cosmid, or double-stranded M13 DNA.

1. Growth of Bacterial Cultures

Plasmids are generally prepared from bacterial cultures grown in the presence of a selective agent such as an antibiotic (1, 3). The yield and quality of the plasmid DNA prepared may depend on a number of factors including plasmid copy number, size of insert, host strain, culture volume, and culture medium.

Plasmid copy number

Plasmids vary widely in their copy number (Table 5), depending on the origin of replication they contain (pMB1 or pSC101 for example) which determines whether they are under relaxed or stringent control; as well as the size of the plasmid and its associated insert. Some plasmids, such as the pUC series and derivatives, have mutations which allow them to reach very high copy numbers within the bacterial cell. Plasmids based on pBR322 and many cosmids are generally maintained at lower copy numbers. Very large plasmids are often maintained at very low copy numbers per cell. **Please note:** the copy number of plasmids and cosmids can be substantially influenced by the cloned insert. For example, a high-copy pUC plasmid may behave like a medium- or low-copy plasmid when containing certain inserts (e.g. very large DNA fragments), resulting in lower DNA yields than expected.

Table 5. Origins of replication and copy numbers of various plasmids and cosmids (1)

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.*

Cosmid copy number

QIAGEN Plasmid Kits are also ideal for purification of cosmid DNA. Due to their relatively large size and slow replication time, cosmids are generally present in low or very low copy numbers in bacterial cells (Table 5). Like plasmids, cosmids vary in copy number, depending on the origin of replication they contain, their size, and the size of insert. Cosmids should be treated as low- or very low-copy plasmids when determining which

QIAGEN-tip to use. In order to obtain good yields of very low-copy cosmids, it is often necessary to use culture volumes much larger than those normally recommended for use on QIAGEN-tips. A few changes in the procedure are necessary to obtain optimal results. See the detailed protocol on page 40. Cosmid DNA prepared with QIAGEN-tips is suitable for all applications including sequencing (manual or automated).

For purification of P1 and BAC DNA using QIAGEN-tips, please contact our technical service groups or your local distributor (see page 79).

Host strains

Most *E. coli* strains can be used successfully to isolate plasmid DNA, although the strain used to propagate a plasmid can have a substantial influence on the quality of the purified DNA. Host strains such as DH1, DH5α™, and C600 yield high-quality DNA with QIAGEN protocols. The slower growing strain XL1-Blue also yields DNA of very high quality which works extremely well for sequencing. 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 (3). In addition, some strains such as JM101, JM110, and HB101 have high levels of endonuclease activity, and yield DNA of lower quality than that prepared from strains such as XL1-Blue, DH1, DH5α, and C600. The methylation and growth characteristics of the host strain can also affect plasmid isolation. If after performing a QIAGEN plasmid preparation, 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 used for cleared lysate preparation, or using the same amount of culture volume but doubling the volumes of Buffers P1, P2, and P3 in order to improve the ratio of biomass to lysis buffers for optimized lysis conditions.

Inoculation

Bacterial cultures for plasmid preparation should always be grown from a single colony picked from a freshly streaked selective plate. Subculturing directly from glycerol stocks, agar stabs, and liquid cultures is poor microbiological practice and may lead to loss of the plasmid. Inoculation from plates that have been stored for a long time may also lead to loss or mutation of the plasmid.

The desired clone should be streaked from a glycerol stock onto a freshly prepared agar plate containing the appropriate selective agent such that single colonies can be isolated. This procedure should then be repeated to ensure that a single colony of an antibiotic-resistant clone can be picked. A single colony should be inoculated into 2–10 ml of LB medium (see page 10) containing the appropriate selective agent and grown for ~8 hours (logarithmic phase). Using a vessel with a volume of at least four times greater than the volume of medium, the starter culture should then be diluted 1/500 to 1/1000 into a larger volume of selective medium, and grown with vigorous shaking (~300 rpm) to saturation (12–16 hours). It is often convenient to grow the starter culture during the day and the larger culture overnight for harvesting the following morning.

Antibiotics

Antibiotic selection should be applied at all stages of growth. Many plasmids in use today do not contain the par locus which ensures that the plasmids segregate equally during cell division in the absence of selective pressure. Daughter cells that do not receive plasmids will replicate much faster than plasmid-containing cells and can quickly take over the culture.

The stability of the selective agent should also be taken into account. Resistance to ampicillin, for example, is mediated by β-lactamase which is encoded by the plasmid-linked *bla* gene and which hydrolyzes ampicillin. Levels of ampicillin in the culture medium are thus continually depleted. This phenomenon is clearly demonstrated on ampicillin plates, where "satellite colonies" appear as the ampicillin is hydrolyzed in the vicinity of a growing colony. It is important to inoculate cultures from freshly prepared plates to ensure that the antibiotic is effective. Ampicillin is also very sensitive to temperature, and should be stored frozen in single-use aliquots. Table 6 gives the concentrations of commonly used antibiotics.

Table 6. Concentrations of commonly used antibiotics

Antibiotic	Stock solutions		Working concentration (dilution)
	Concentration	Storage	
Ampicillin (sodium salt)	50 mg/ml in H_2O	–20°C	100 µg/ml (1/500)
Chloramphenicol	34 mg/ml in ethanol	–20°C	170 µg/ml (1/200)
Kanamycin	10 mg/ml in H_2O	–20°C	50 µg/ml (1/200)
Streptomycin	10 mg/ml in H_2O	–20°C	50 µg/ml (1/200)
Tetracycline HCl	5 mg/ml in ethanol	–20°C	50 µg/ml (1/100)

Culture media

QIAGEN protocols are 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 (see page 60). We advise harvesting cultures after approximately 12–16 hours of growth, which typically is the transition from logarithmic into stationary growth phase. At this time, the ratio of plasmid DNA to RNA is higher than during the logarithmic phase. Also, the DNA is not yet degraded due to overaging of the culture, as in the later stationary phase. Please note the maximum recommended culture volumes given at the beginning of each protocol.

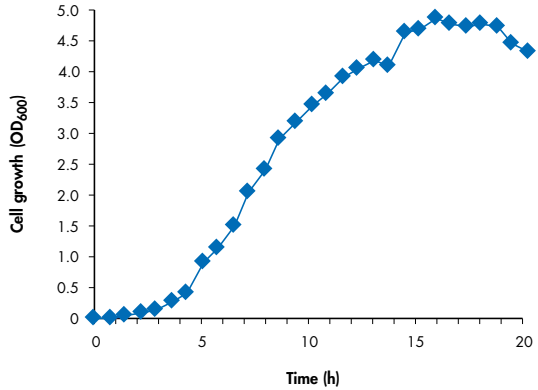


Figure 6. *Growth curve of* E. coli *in LB medium. Host strain: DH5α; plasmid: pUC21. High OD_{600} readings were calculated by diluting the sample to enable photometric measurement in the linear range between 0.1–0.5 OD_{600}.*

Several of the current bacteria strains can grow to very high cell densities. It is best to assess the cell density of the culture and reduce the culture volumes accordingly or increase the volumes of lysis buffers P1, P2 and P3, if necessary. A high ratio of biomass to lysis buffers will result in poor lysis conditions and subsequently low DNA yield and purity.

If working with low-copy vectors, it may be beneficial to increase the lysis buffer volumes in order to increase the efficiency of alkaline lysis, and thereby the DNA yield (Figure 7). In case additional Buffers P1, P2, and P3 are needed, their compositions are provided on page 68. Alternatively, the buffers may be purchased separately (see page 78).

It is not recommended to use super rich growth media such as TB (terrific broth) or 2x YT for most commonly used high-copy plasmids. Although TB or 2x YT have the obvious advantage of producing more bacteria (2–5 times), this does not necessarily lead to greater yields or higher-quality DNA.

If rich media must be used, the culture volume should be reduced to match the recommended cell biomass, which in turn should correspond to the capacity of the QIAGEN-tip used. If the culture volume used is too high, alkaline lysis will be inefficient, resulting in lower yield than expected. Furthermore, the excessive viscosity of the lysate will require vigorous mixing, resulting in shearing of bacterial genomic DNA and subsequent contamination of the plasmid DNA.

Measuring cell density

Photometric measurements of cell density can vary between different spectrophotometers. The optical density reading of a bacterial culture is a measure of the light scattering, which varies depending on the distance between the sample and the detector.

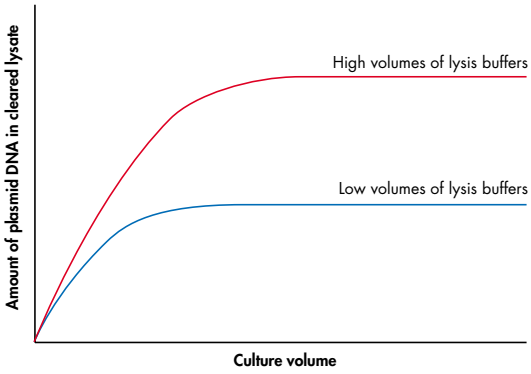


Figure 7. *The effect of lysis buffer volumes on the amount of DNA in the cleared lysate.*

Calibration of each individual spectrophotometer is required to facilitate accurate conversion of OD_{600} measurements into the number of cells per ml. 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 ml, which is then set in relation to the measured OD_{600} values.

Pellet wet weight

If spectrophotometric measurement of the cell density or calibration of the photometer is not possible, another way of estimating the amount of cell harvest is by assessment of the pellet wet weight. Typically, a 1-liter, overnight shaker-culture of *E.coli* with a cell density of 3–4 x 10^9/ml corresponds to a pellet wet weight of approximately 3 g/liter.

Chloramphenicol amplification

The copy numbers of the current generation of plasmids are so high that selective amplification in the presence of chloramphenicol is not necessary to achieve high yields. However, when low-copy-number plasmids containing the pMB1 or ColE1 origin of replication are prepared, the yield can be improved by adding chloramphenicol (170 mg/liter) to amplify the copy number (1). Cultures of bacteria containing low-copy-number plasmids amplified in the presence of chloramphenicol should be treated as if they contain high-copy-number plasmids when choosing the appropriate culture volumes for the QIAGEN-tip to be used.

Purification of M13 replicative form

The replicative form of bacteriophage M13 behaves like a low-copy-number plasmid and can be purified using QIAGEN or QIAfilter Plasmid Kits (page 45). The only modification of the procedure is the introduction of a wash step before the cells are lysed. This step

removes all traces of the phage-rich culture supernatant from the bacterial pellet and prevents contamination of the double-stranded M13 RF with single-stranded phage DNA.

In vitro transcription

Plasmid DNA preparations are free of any detectable proteins or other contaminants when purified on QIAGEN-tips according to the recommended protocol. DNA purified using QIAGEN, QIAfilter, or EndoFree Plasmid Kits gives excellent results with in vitro transcription experiments. Although a high level of RNase A is employed at the beginning of the procedure, it is removed efficiently by potassium dodecyl sulfate precipitation and subsequent washing with Buffer QC.

It is possible, although not necessary, to omit RNase A from the procedure when purifying DNA for in vitro transcription. In this case, increasing the volume of the wash buffer (QC) is recommended (e.g., for a Midi preparation on a QIAGEN-tip 100, use at least 2 x 30 ml of Buffer QC instead of 2 x 10 ml).

2. Key Steps in the Plasmid Purification Protocols

After lysis of bacteria under alkaline conditions, the lysate is applied under defined salt conditions to the QIAGEN-tip. Plasmid DNA is selectively bound and purified from RNA, proteins, and other cellular contaminants.

Preparation of the cell lysate

DNA yield depends on the quality of the cell lysate used. Preparation of a cleared cell lysate is therefore a critical step in the QIAGEN purification procedure, which has been carefully designed to provide ideal lysis conditions.

After harvesting and resuspension, the bacterial cells are lysed in NaOH–SDS (Buffer P2) in the presence of RNase A (2, 4). SDS solubilizes the phospholipid and protein components of the cell membrane, leading to lysis and release of the cell contents. NaOH denatures the chromosomal and plasmid DNAs, as well as proteins. The optimized lysis time allows maximum release of plasmid DNA from the cell without release of chromosomal DNA, while minimizing the exposure of the plasmid to denaturing conditions. Long exposure to alkaline conditions may cause the plasmid to become irreversibly denatured. This denatured form of the plasmid runs faster on agarose gels and is resistant to restriction enzyme digestion (see Figure 4, page 46).

The lysate is neutralized by the addition of acidic potassium acetate (Buffer P3). The high salt concentration causes SDS to precipitate, and the denatured proteins, chromosomal DNA, and cellular debris become trapped in salt–detergent complexes. Plasmid DNA, being smaller and covalently closed, renatures correctly and remains in solution. Since any SDS remaining in the lysate will inhibit binding of DNA to QIAGEN Resin, the solution must be thoroughly but gently mixed to ensure complete precipitation of the detergent.

Separation of plasmid from chromosomal DNA is based on coprecipitation of the cell-wall-bound chromosomal DNA with the insoluble complexes containing salt, detergent, and protein. Plasmid DNA remains in the clear supernatant. Vigorous treatment during the lysis procedure will shear the bacterial chromosome, leaving free chromosomal DNA fragments in the supernatant. Since chromosomal fragments are chemically indistinguishable from plasmid DNA under the conditions used, the two species will not be separated on QIAGEN Resin and will elute under the same salt conditions.

RNase A, which is added at the beginning of the procedure, digests the liberated RNA efficiently during the alkaline lysis. The resulting RNA fragments do not bind to QIAGEN Resin under the salt and pH conditions present in the lysate.

The precipitated debris is removed by high-speed centrifugation or by use of a QIAfilter Cartridge, producing a cleared lysate for loading onto the QIAGEN-tip. It is important that the lysate is clear at this stage to ensure good flow rates and, ultimately, to obtain protein-free plasmid DNA preparations.

Clearing of bacterial lysates using QIAfilter Cartridges

QIAfilter Cartridges are special filtration units designed to replace the centrifugation step after alkaline lysis of bacterial cells. After cultures are pelleted, bacterial cells are lysed in NaOH–SDS, neutralized by the addition of acidic potassium acetate, and incubated directly in the QIAfilter Cartridge. The lysate is cleared in a matter of seconds by passing the liquid through the filter. Insoluble complexes containing chromosomal DNA, salt, detergent, and proteins, which form during the neutralization step are completely removed. QIAfilter Cartridges clear bacterial lysates more efficiently than conventional centrifugation. In addition, small-sized SDS precipitates which cannot be separated by centrifugation are completely removed by the QIAfilter process.

DNA binding and washing on the QIAGEN-tip

The cleared lysate is loaded onto a pre-equilibrated QIAGEN-tip by gravity flow. The salt and pH conditions of the lysate and the superior selectivity of the QIAGEN Resin ensure that only plasmid DNA binds, while degraded RNA, cellular proteins, and metabolites are not retained and appear in the flow-through fraction.

The QIAGEN-tip is then washed with medium-salt buffer (Buffer QC) which completely removes any remaining contaminants, such as traces of RNA and protein (e.g. RNase A), without affecting the binding of the plasmid DNA (see Figure 4 on page 46). Buffer QC also disrupts nonspecific interactions, and allows removal of nucleic acid-binding proteins without the use of phenol. The low concentration of alcohol in the wash buffer eliminates nonspecific hydrophobic interactions, further enhancing the purity of the bound DNA. The plasmid DNA is then efficiently eluted from the QIAGEN-tip with high-salt buffer (Buffer QF or QN). For further information about QIAGEN Anion-Exchange Resin, see Appendix B, pages 69–71.

Desalting and concentration

The eluted plasmid DNA is desalted and concentrated by isopropanol precipitation. Precipitation is carried out at room temperature to minimize coprecipitation of salt. After centrifugation, the DNA pellet is washed with 70% ethanol to remove residual salt and to replace the isopropanol with ethanol, which is more volatile and easily removed. The purified DNA is briefly air-dried and redissolved in a small volume of TE, pH 8.0 or Tris·Cl, pH 8.5, and is ready for use in transfection, sequencing, labeling, cloning, or any other experimental procedure.

3. 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 8). A single *E. coli* cell contains about 2 million LPS molecules (5, 6), each consisting of a hydrophobic lipid A moiety, a complex array of sugar residues and negatively charged phosphate groups (Figure 9). 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.

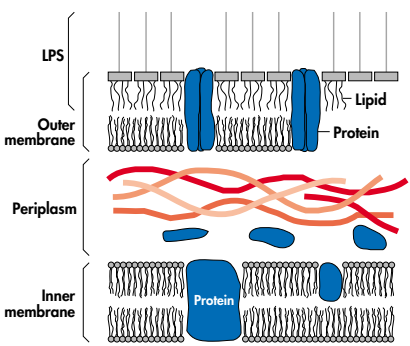


Figure 8. *Schematic diagram of the envelope of* E. coli.

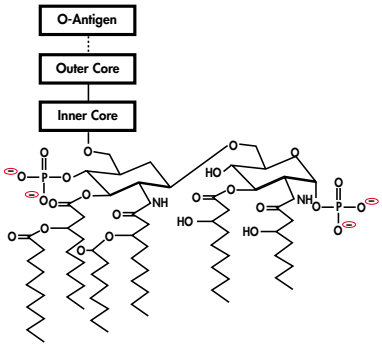


Figure 9. *Schematic diagram of the endotoxin molecule.*

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 (7, 8). QIAGEN Plasmid Kits and 2x 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 7).

Table 7. 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 (8), and increased endotoxin levels lead to sharply reduced transfection efficiencies (9). 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 (10). 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 (11, 12). It is important to make sure that plasticware, media, sera, and plasmid DNA are free of LPS contamination in order to avoid misinterpretation of experimental results.

Removal of endotoxins

The patented EndoFree Plasmid Procedure (pages 30–39) 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. 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

In order to avoid recontamination of plasmid DNA after initial endotoxin removal, we recommend using only new plasticware which is certified to be 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. For further reading on endotoxin removal, please refer to the appropriate literature such as reference 13 on page 72.

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 TE buffer for resuspension.

Appendix A

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	room temp.
Buffer P3 (Neutralization Buffer)	3.0 M potassium acetate, pH 5.5	room temp. or 2–8°C
Buffer FWB (QIAfilter Wash Buffer)	750 mM NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol	room temp.
Buffer QBT (Equilibration Buffer)	750 mM NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol; 0.15% Triton® X-100	room temp.
Buffer QC (Wash Buffer)	1.0 M NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol	room temp.
Buffer QF (Elution Buffer)	1.25 M NaCl; 50 mM Tris·Cl, pH 8.5; 15% isopropanol	room temp.
Buffer QN (Elution Buffer)	1.6 M NaCl; 50 mM MOPS, pH 7.0; 15 % isopropanol	room temp.
TE	10 mM Tris·Cl, pH 8.0; 1 mM EDTA	room temp.
STE	100 mM NaCl; 10 mM Tris·Cl, pH 8.0; 1 mM EDTA	room temp.

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-HCl 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 dH_2O. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 liter with dH_2O. Add 100 mg RNase A per liter of P1.

P2: Dissolve 8.0 g NaOH pellets in 950 ml dH_2O, 50 ml 20% SDS solution. The final volume should be 1 liter.

P3: Dissolve 294.5 g potassium acetate in 500 ml dH_2O. Adjust the pH to 5.5 with glacial acetic acid (~110 ml). Adjust the volume to 1 liter with dH_2O.

FWB: Dissolve 43.83g NaCl, 10.46 g MOPS (free acid) in 800 ml dH_2O. Adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol and adjust the volume to 1 liter with dH_2O.

QBT: Dissolve 43.83 g NaCl, 10.46 g MOPS (free acid) in 800 ml dH_2O. Adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol and 15 ml 10% Triton X-100 solution. Adjust the volume to 1 liter with dH_2O.

QC: Dissolve 58.44 g NaCl and 10.46 g MOPS (free acid) in 800 ml dH_2O. Adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with dH_2O.

QF: Dissolve 73.05 g NaCl and 6.06 g Tris base in 800 ml dH_2O and adjust the pH to 8.5 with HCl. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with dH_2O.

QN: Dissolve 93.50 g NaCl and 10.46 g MOPS (free acid) in 800 ml dH_2O and adjust the pH to 7.0 with NaOH. Add 150 ml pure isopropanol. Adjust the volume to 1 liter with dH_2O.

STE: Dissolve 5.84 g NaCl, 1.21 g Tris base, and 0.37 g $Na_2EDTA{\cdot}2H_2O$ in 800 ml dH_2O. Adjust the pH to 8.0 with HCl. Adjust the volume to 1 liter with dH_2O.

Preparation of LB medium

Dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 800 ml dH_2O. Adjust the pH to 7.0 with 1 N NaOH. Adjust the volume to 1 liter with dH_2O. Sterilize by autoclaving.

Appendix B

General information about QIAGEN Anion-Exchange Resin

QIAGEN-tips contain a unique, patented anion-exchange resin which eliminates the need for expensive equipment and reagents such as ultracentrifuges, HPLC/FPLC®, or CsCl. Toxic and mutagenic substances such as phenol, chloroform, and ethidium bromide are also not required.

Plasmid purification on QIAGEN Resin is based on the interaction between negatively charged phosphates of the DNA backbone and positively charged DEAE groups on the surface of the resin (Figure 10). The salt concentration and pH conditions of the buffers used determine whether DNA is bound or eluted from the column. The key advantage of QIAGEN Anion-Exchange Resin arises from its exceptionally high charge density. The resin consists of defined silica beads with a particle size of 100 µm, a large pore size, and a hydrophilic surface coating. The large surface area allows dense coupling of the DEAE groups. Plasmid DNA remains tightly bound to the DEAE groups over a wide range of salt concentrations (Figure 11). Impurities such as RNA, protein, carbohydrates, and small metabolites are washed from QIAGEN Resin with medium-salt buffers, while plasmid DNA remains bound until eluted with a high-salt buffer. The separation range of QIAGEN Resin is extremely broad, extending from 0.1 M to 1.6 M salt (Figure 11), and DNA can be efficiently separated from RNA and other impurities. In contrast, conventional anion-exchangers, based on cellulose, dextran or agarose, have separation ranges only up to 0.4 M salt, so that binding and elution of all substances is limited to a narrow range of salt concentrations. This means that the elution peaks of proteins, RNA, and DNA overlap extensively with one another, and a satisfactory separation cannot be achieved. Thus the separation and purification qualities of QIAGEN Resin as well as its ease of use surpass those of conventional anion-exchange resins.

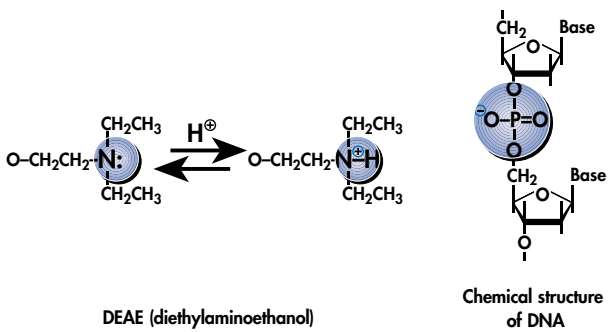


Figure 10. *Chemical structure of positively charged DEAE groups of QIAGEN Resin, and negatively charged groups of the DNA backbone which interact with the resin.*

Purity and biological activity

Nucleic acids prepared on QIAGEN Resin are of equivalent or superior purity to nucleic acids prepared by two rounds of purification on CsCl gradients. DNA prepared using QIAGEN-tips has been tested with restriction endonucleases, polymerases (including *Taq* DNA polymerase), DNA ligases, phosphatases, and kinases. Subsequent procedures such as transfection, transformation, sequencing, cloning, and in vitro transcription and translation proceed with optimal efficiency.

Capacity and recovery

The names of the different QIAGEN-tips indicate the binding capacities (in µg) of the columns for double-stranded plasmid DNA, as determined with purified pUC18 DNA. QIAGEN-tip 100, for example, has a binding capacity of 100 µg of plasmid DNA.

QIAGEN Resin has different binding capacities for different classes of nucleic acids. The capacity of QIAGEN Resin for RNA, for example, is twice that for plasmid DNA. Conversely, large nucleic acids, such as lambda, cosmids and genomic DNA, are bound at a slightly lower capacity than plasmid DNA. This relationship between the binding capacity of the QIAGEN Resin and the size of the nucleic acids being prepared must be taken into account when calculating expected yields.

Stability

QIAGEN Resin is stable for up to six hours after equilibration. Beyond this time, the separation characteristics of the resin will begin to change, and it will no longer be effective. QIAGEN-tips may be reused within six hours for the same sample by re-equilibrating the resin with Buffer QBT after the first elution. QIAGEN Resin will not function in the presence of anionic detergents such as SDS, or at a pH less than 4.0.

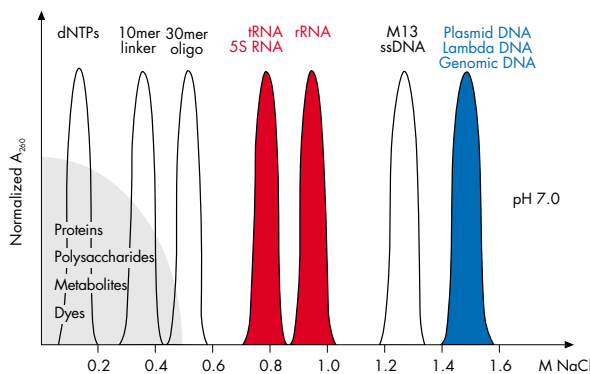


Figure 11. *Separation of nucleic acids at neutral pH on QIAGEN Anion-Exchange Resin.*

Buffers

The binding, washing, and elution conditions for QIAGEN Resin are strongly influenced by pH. Figure 12 shows the influence of pH on the salt concentration required for elution of various types of nucleic acids. Deviations from the appropriate pH values of the buffers at a given salt concentration may result in losses of the desired nucleic acid.

Buffers, such as MOPS, sodium phosphate, Tris·Cl and sodium acetate can be used at the indicated pH. MOPS (3-[N-morpholino]propanesulfonic acid, pKa 7.2) is frequently the buffer of choice in QIAGEN protocols, since it has a higher buffering capacity at pH 7.0 than sodium phosphate, Tris·Cl or sodium acetate buffers.

SDS and other anionic detergents interfere with the binding of nucleic acids to QIAGEN Resin by competing for binding to the anion-exchange groups. If SDS is used during sample preparation, it must be removed through steps such as potassium acetate precipitation or alcohol precipitation prior to column application. SDS removal steps are incorporated into the QIAGEN protocols described in this manual.

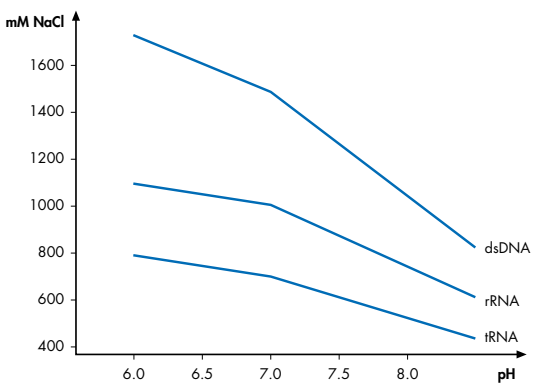


Figure 12. *Elution points of different nucleic acids from QIAGEN Resin as a function of pH and NaCl concentration.*

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Product Use Limitations

QIAGEN, QIAfilter, and EndoFree Plasmid Kits are developed, designed and sold for research purposes only. They are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department. We will credit your account or exchange the product — as you wish.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor.

Kit Contents

QIAGEN Plasmid Kits

QIAGEN Plasmid Kit	Starter Kit	Midi (25)	Midi (50)	Midi (100)	Plasmid Buffer Set
Catalog No.	**12129**	**12143**	**12144**	**12145**	**19046**
QIAGEN-tip 20	10	–	–	–	–
QIAGEN-tip 100	3	25	50	100	–
QIAGEN-tip 500	1	–	–	–	–
Buffer P1	1 x 40 ml	1 x 110 ml	1 x 220 ml	1 x 440 ml	1 x 110 ml
Buffer P2	1 x 40 ml	1 x 110 ml	1 x 220 ml	1 x 440 ml	1 x 110 ml
Buffer P3	1 x 40 ml	1 x 110 ml	1 x 220 ml	1 x 440 ml	1 x 110 ml
Buffer QBT	1 x 40 ml	1 x 110 ml	1 x 220 ml	1 x 440 ml	1 x 110 ml
Buffer QC	2 x 110 ml	3 x 190 ml	4 x 220 ml, 1 x 240 ml	3 x 750 ml	6 x 120 ml
Buffer QF	1 x 60 ml	1 x 170 ml	1 x 110 ml, 1 x 170 ml	2 x 280 ml	2 x 85 ml
RNase A*	1 x 4 mg	1 x 11 mg	1 x 22 mg	1 x 44 mg	1 x 11 mg
Handbook (QIAGEN Plasmid Purification)	1	1	1	1	1
Handbook (QIAGEN Plasmid Mini)	1	–	–	–	1

QIAGEN Plasmid Kit	Maxi (10)	Maxi (25)	Maxi (100)	Mega (5)	Mega (25)	Giga (5)
Catalog No.	**12162**	**12163**	**12165**	**12181**	**12183**	**12191**
QIAGEN-tip 500	10	25	4 x 25	–	–	–
QIAGEN-tip 2500	–	–	–	5	25	–
QIAGEN-tip10000	–	–	–	–	–	5
Buffer P1	1 x 110 ml	1 x 280 ml	4 x 280 ml	2 x 140 ml	2 x 700 ml	1 x 700 ml
Buffer P2	1 x 110 ml	1 x 280 ml	4 x 280 ml	2 x 140 ml	2 x 700 ml	1 x 700 ml
Buffer P3	1 x 110 ml	1 x 280 ml	4 x 280 ml	2 x 140 ml	2 x 700 ml	1 x 700 ml
Buffer QBT	1 x 110 ml	1 x 280 ml	4 x 280 ml	1 x 200 ml	2 x 500 ml	1 x 400 ml
Buffer QC	3 x 240 ml	4 x 425 ml	16 x 425 ml	5 x 220 ml	5 x 1000 ml, 1 x 500 ml	3 x 1000 ml, 1 x 500 ml
Buffer QF	1 x 170 ml	1 x 420 ml,	4 x 420 ml	1 x 200 ml	2 x 500 ml	1 x 400 ml
RNase A*	1 x 11 mg	1 x 28 mg	4 x 28 mg	2 x 14 mg	2 x 70 mg	1 x 70 mg
Handbook	1	1	1	1	1	1

** Provided in a 100 mg/ml solution.*

QIAfilter Plasmid Kits

QIAfilter Plasmid Kit	Midi (25)	Midi (100)	Maxi (10)	Maxi (25)	Mega (5)	Giga (5)
Catalog No.	**12243**	**12245**	**12262**	**12263**	**12281**	**12291**
QIAGEN-tip 100	25	100	–	–	–	–
QIAGEN-tip 500	–	–	10	25	–	–
QIAGEN-tip 2500	–	–	–	–	5	–
QIAGEN-tip 10000	–	–	–	–	–	5
QIAfilter Midi Cartridges	25	100	–	–	–	–
QIAfilter Maxi Cartridges	–	–	10	25	–	–
QIAfilter Mega-Giga Cartridges*	–	–	–	–	5	5
Caps for QIAfilter	25	100	10	25	–	–
Buffer P1	1 x 110 ml	4 x 110 ml	1 x 110 ml	1 x 280 ml	2 x 140 ml	1 x 700 ml
Buffer P2	1 x 110 ml	4 x 110 ml	1 x 110 ml	1 x 280 ml	2 x 140 ml	1 x 700 ml
Buffer P3	1 x 110 ml	4 x 110 ml	1 x 110 ml	1 x 280 ml	2 x 140 ml	1 x 700 ml
Buffer FWB	–	–	–	–	2 x 140 ml	2 x 140 ml
Buffer QBT	1 x 110 ml	4 x 110 ml	1 x 110 ml	1 x 280 ml	1 x 200 ml	1 x 400 ml
Buffer QC	3 x 190 ml	12 x 190 ml	3 x 240 ml	2 x 850 ml	5 x 220 ml	3 x 1000 ml, 1 x 500 ml
Buffer QF	1 x 170 ml	4 x 170 ml	1 x 170 ml	1 x 420 ml	1 x 200 ml	1 x 400 ml
RNase A†	1 x 11 mg	4 x 11 mg	1 x 11 mg	1 x 28 mg	2 x 14 mg	1 x 70 mg
Handbook	1	1	1	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 100 mg/ml solution.*

EndoFree Plasmid Kits

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	1 x 110 ml	2 x 140 ml	1 x 700 ml
Buffer P2	1 x 110 ml	2 x 140 ml	1 x 700 ml
Buffer P3	1 x 110 ml	2 x 140 ml	1 x 700 ml
Buffer FWB	–	2 x 140 ml	2 x 140 ml
Buffer QBT	1 x 110 ml	1 x 200 ml	1 x 400 ml
Buffer QC	3 x 240 ml	5 x 220 ml	3 x 1000 ml, 1 x 500 ml
Buffer QN	1 x 170 ml	1 x 200 ml	1 x 400 ml
Buffer ER	1 x 30 ml	1 x 80 ml	1 x 200 ml
Buffer TE	1 x 30 ml	1 x 110 ml	1 x 110 ml
Endotoxin-free H_2O for 70% ethanol	1 x 17 ml	1 x 17 ml	1 x 17 ml
RNase A†	1 x 11 mg	2 x 14 mg	1 x 70 mg
Handbook	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 100 mg/ml solution.

Ordering Information

Product	Contents	Cat. No.
QIAGEN Plasmid Kits		
QIAGEN Plasmid Midi Kit (25)	25 QIAGEN-tip 100, Reagents, Buffers	12143
QIAGEN Plasmid Midi Kit (50)	50 QIAGEN-tip 100, Reagents, Buffers	12144
QIAGEN Plasmid Midi Kit (100)	100 QIAGEN-tip 100, Reagents, Buffers	12145
QIAGEN Plasmid Maxi Kit (10)	10 QIAGEN-tip 500, Reagents, Buffers	12162
QIAGEN Plasmid Maxi Kit (25)	25 QIAGEN-tip 500, Reagents, Buffers	12163
QIAGEN Plasmid Maxi Kit (100)	100 QIAGEN-tip 500, Reagents, Buffers	12165
QIAGEN Plasmid Mega Kit (5)	5 QIAGEN-tip 2500, Reagents, Buffers	12181
QIAGEN Plasmid Mega Kit (25)	25 QIAGEN-tip 2500, Reagents, Buffers	12183
QIAGEN Plasmid Giga Kit (5)	5 QIAGEN-tip 10000, Reagents, Buffers	12191
QIAfilter Plasmid Kits		
QIAfilter Plasmid Midi Kit (25)	25 QIAGEN-tip 100, Reagents, Buffers, 25 QIAfilter Midi Cartridges	12243
QIAfilter Plasmid Midi Kit (100)	100 QIAGEN-tip 100, Reagents, Buffers, 100 QIAfilter Midi Cartridges	12245
QIAfilter Plasmid Maxi Kit (10)	10 QIAGEN-tip 500, Reagents, Buffers, 10 QIAfilter Maxi Cartridges	12262
QIAfilter Plasmid Maxi Kit (25)	25 QIAGEN-tip 500, Reagents, Buffers, 25 QIAfilter Maxi Cartridges	12263
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

Ordering Information

Product	Contents	Cat. No.
EndoFree Plasmid Kits		
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
Transfection Products		
SuperFect Transfection Reagent (1.2 ml)	For 40 transfections in 60-mm dishes or 160 transfections in 12-well plates	301305
SuperFect Transfection Reagent (4 x 1.2 ml)	For 160 transfections in 60-mm dishes or 640 transfections in 12-well plates	301307
Effectene Transfection Reagent (1 ml)	1 ml Effectene Reagent; Enhancer, Buffer, for 40 transfections in 60-mm dishes or 160 transfections in 12-well plates	301425
Accessories		
QIArack 2	1 rack for 8 x QIAGEN-tip 100, and 4 x QIAGEN-tip 500	19014
RNase A	250 mg (70 U/mg; 100 mg/ml); for 2.5 liters of working 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 H_2O, RNase A; for 10 plasmid mega- or 5 gigapreps (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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