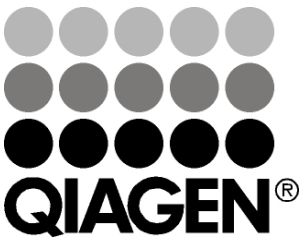
January 2008

# CompactPrep® Plasmid Mega/Giga Purification Handbook

For preparation of molecular biology grade plasmid DNA from *E. coli*

CompactPrep Plasmid Mega and Giga Kits




Sample & Assay Technologies

# QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

**QIAGEN sets standards in**:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com .

# Contents

## Kit Contents

| | CompactPrep Plasmid Mega Kit (5) | CompactPrep Plasmid Giga Kit (5) |
|---|---|---|
| **Catalog no.** | **12781** | **12791** |
| **Number of preps** | **5** | **5** |
| CompactPrep Spin Columns | 5 Mega Columns | 5 Giga Columns |
| QIAfilter Mega-Giga Cartridges | 5 | 5 |
| Tube Extenders | 5 | 5 |
| Collection Tubes (50 ml) | 10 | 10 |
| Buffer P1 | 280 ml | 700 ml |
| Buffer P2 | 280 ml | 700 ml |
| Buffer S3 | 280 ml | 2 x 280 ml |
| Buffer BB | 280 ml | 2 x 280 ml |
| Buffer CPW | 425 ml | 425 ml |
| Buffer PE (concentrate) | 100 ml | 100 ml |
| Buffer EB | 55 ml | 2 x 55 ml |
| RNase A (100 mg/ml) | 28 mg | 70 mg |
| LyseBlue | 280 µl | 700 µl |
| Handbook | 1 | 1 |

## Storage

CompactPrep Plasmid Kits should be stored dry at room temperature (15–25°C).

CompactPrep Mega and Giga spin columns can be stored for up to 12 months without any reduction in performance and quality. After adding RNase A, Buffer P1 should be stored at 2–8°C and is stable for 6 months. Other buffers, RNase A stock solution and QIAfilter cartridges can be stored for 2 years at room temperature (15–25°C).

## Quality Control

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

## Product Use Limitations

CompactPrep Plasmid Mega and Giga Kits are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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

## 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 or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

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 (see back cover or visit www.qiagen.com ).

## Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding CompactPrep Plasmid Kits or QIAGEN products in general, please do not hesitate to contact us. QIAGEN customers are 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 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 see our Technical Support center at www.qiagen.com/goto/TechSupportCenter or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com ).

Comprehensive background information on plasmid preparation procedures and common plasmid applications — in addition to kit selection guides, frequently asked questions, and information about our purification technologies — can be found on our plasmid web page www.qiagen.com/goto/plasmidinfo .

# Safety Information

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

**Buffer BB**

Contains cetrimonium bromide: dangerous for the environment. Risk and safety phrases:* R52/53

**Buffer P2**

Contains sodium hydroxide: irritant. Risk and safety phrases:* R36/38, S13-26-36-46

**RNase A**

Contains ribonuclease: sensitizer. Risk and safety phrases:* R42/43, S23-24-26-36/37

**24-hour emergency information**

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R36/38: Irritating to eyes and skin; R42/43: May cause sensitization by inhalation and skin contact; R52/53: Harmful to aquatic organisms and may cause long-term adverse effects to the aquatic environment; S13: Keep away from food, drink, and animal feedingstuffs; S23: Do not breathe spray; S24: Avoid contact with skin; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; S36/37: Wear suitable protective clothing and gloves; S46: If swallowed seek medical advice immediately and show this container or label.

# Introduction

QIAGEN CompactPrep Plasmid Kits provide a novel method for very fast and easy large-scale plasmid preparation. The procedure can be performed in 40 minutes (CompactPrep Mega) or 50 minutes (CompactPrep Giga) and uses a vacuum pump and centrifuge. The design and unique binding chemistry of the CompactPrep Mega and Giga columns allow processing of up to 12 CompactPrep Mega or Giga preparations in parallel on the QIAvac 24 Plus with an appropriate waste container.

The procedure is based on a novel, non-chaotropic, silica-binding chemistry (patent pending). After lysate clearing, the process follows a simple bind-wash-elute procedure. The resulting highly concentrated DNA is immediately ready for use in subsequent applications.

CompactPrep Plasmid Kits provide molecular biology grade DNA, highly suited for routine applications such as enzymatic modification, cloning, sequencing, and for transfection into most cell lines (including more sensitive cell lines such as Huh-7). A higher DNA quality may be required for transfection into highly sensitive cell lines. QIAGEN anion-exchange–based and EndoFree plasmid kits offer the highest possible DNA quality and are ideally suited for all sensitive applications.

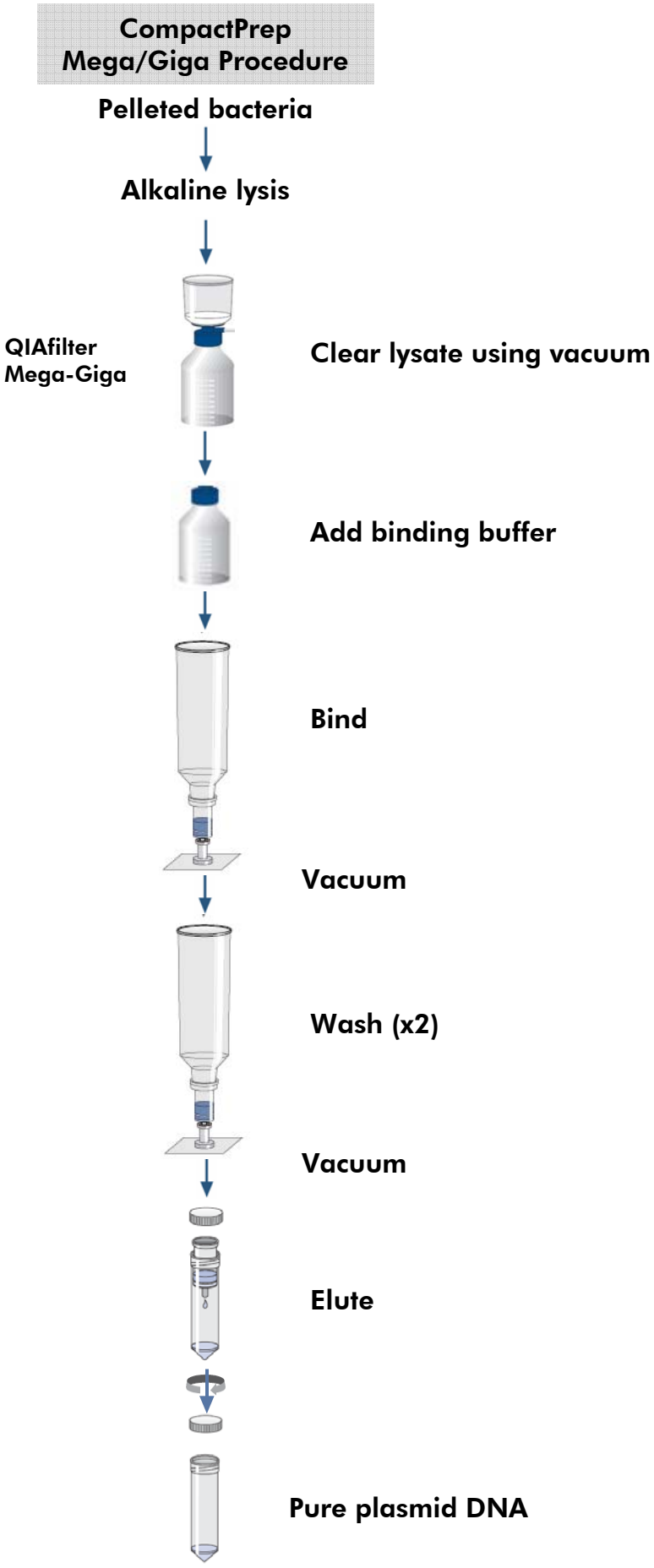
QIAGEN offers the most comprehensive portfolio of tailored plasmid purification kits for any scale, throughput, or downstream application. Select the optimum kit for your requirements by visiting our online selection guide at www.qiagen.com/products/plasmid/selectionguide .

## Principle and procedure

CompactPrep Plasmid Kit protocols are based on a modified alkaline lysis procedure. After neutralization, the lysate is cleared using a QIAfilter Mega-Giga cartridge. A non-chaotropic binding buffer (Buffer BB) is added to the cleared lysate in order to optimize binding of plasmid DNA to the silica membrane of the CompactPrep column. A vacuum manifold is used to draw the cleared lysate and subsequent wash buffers through the CompactPrep column. After two wash steps and drying of the membrane, DNA is eluted in low volumes (1 ml for CompactPrep Mega; 5 ml for CompactPrep Giga) of elution buffer by centrifugation. The highly concentrated DNA (up to >1 µg/µl) is immediately ready for use without the need for further alcohol precipitation.

### LyseBlue reagent

Use of LyseBlue is optional and is not required to successfully perform plasmid preparations. See “Using LyseBlue reagent” on page 15.


CompactPrep
Mega/Giga Procedure
Pelleted bacteria
Alkaline lysis
QIAfilter
Mega-Giga
Clear lysate using vacuum
Add binding buffer
Bind
Vacuum
Wash (x2)
Vacuum
Elute
Pure plasmid DNA

## Equipment and Reagents to Be Supplied by User

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

- Standard microbiological equipment for growing and harvesting bacteria (e.g., inoculating loop, culture tubes and flasks, 37°C shaking incubator, and centrifuge with rotor and tubes or bottles for harvesting cells).
- 96–100% ethanol
- Centrifuge with swing-out rotor and adapters for 50 ml tubes
- Vacuum manifold (e.g., QIAvac 24 Plus, cat. no. 19413)
- VacValves (cat. no. 19408)
- QIAvac Holder (cat. no. 19418)
- For parallel processing of more than one CompactPrep Mega preparation or for CompactPrep Giga preparations on the QIAvac 24 Plus: Connecting system (cat. no. 19419)or 10 liter or 20 liter vacuum bottles with tubing (see Appendix A, page 27)
- Vacuum pump (e.g., QIAGEN Vacuum Pump, cat. no. 84020)
- 1 liter 45 mm-neck vacuum resistant glass bottles (e.g., Schott, cat. no. 2181054) for operating the QIAfilter Mega-Giga Cartridges. Appropriate tubing to connect the cartridges to the vacuum source.

# Important Notes

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

### Plasmid/cosmid copy number

Plasmids and cosmids vary in copy number, depending on the origin of replication they contain, their size, and the size of insert. Protocols for both high- and low-copy number plasmids are provided. For more details, visit our plasmid resource page at www.qiagen.com/goto/plasmidinfo and click on the link "General Considerations for Optimal Results".

### Host strains

The strain used to propagate a plasmid can have a substantial influence on quality of the purified DNA. Host strains such as DH1, DH5α®, and C600 yield high-quality DNA with QIAGEN protocols. The slower growing strain XL1-Blue also yields DNA of very high quality.

Strain HB101 and its derivatives, such as TG1 and the JM100 series, contain large amounts of carbohydrates that are released during lysis and can inhibit enzyme activities if not completely removed. In addition, some strains, such as JM101, JM110, and HB101, have high levels of endonuclease activity and yield DNA of lower quality.

If the quality of purified DNA is not as expected, a change of host strain should be considered. If difficulty is encountered with strains such as TG1 and Top10F, we recommend reducing the amount of culture volume or doubling the volumes of P1, P2, S3, and BB in order to improve the ratio of biomass to lysis buffers for optimized lysis conditions.

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

| DNA construct | Origin of replication | Copy number | Classification |
|---|---|---|---|
| **Plasmids** | | | |
| pUC vectors | pMB1* | 500–700 | High-copy |
| pBluescript® vectors | ColE1 | 300–500 | High-copy |
| pGEM® vectors | pMB1* | 300–400 | High-copy |
| pTZ vectors | pMB1* | >1000 | High-copy |
| pBR322 and derivatives | pMB1* | 15–20 | Low-copy |
| pACYC and derivatives | P15A | 10–12 | Low-copy |
| pSC101 and derivatives | pSC101 | ~5 | Very-low-copy |
| **Cosmids** | | | |
| SuperCos | ColE1 | 10–20 | Low-copy |
| pWE15 | ColE1 | 10–20 | Low-copy |

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

## Culture media

QIAGEN plasmid purification protocols are optimized for use with cultures grown in Luria Bertani (LB) medium to a cell density of approximately 3–4 x $10^9$ cells/ml, which typically corresponds to a pellet wet weight of approximately 3 g/liter of medium. Please note that a number of slightly different LB culture broths, containing different concentrations of NaCl, are commonly used. We recommend growing cultures in LB medium containing 10 g NaCl per liter (Table 2, page 13) to obtain the highest plasmid yields.

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

**Table 2. Composition of LB medium**

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

### Preparation of LB medium

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

### Culture volume

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

The protocol for CompactPrep Mega and Giga Plasmid Kits is optimized for use with cultures grown in LB medium, grown to a cell density of approximately 3–4 x $10^9$ cells/ml. We advise harvesting cultures after approximately 12–16 hours of growth, which typically is the transition from logarithmic into stationary growth phase. It is best to assess the cell density of the culture and, if it is too high, to reduce the culture volumes accordingly, or for CompactPrep Mega, increase the volumes of Buffers P1, P2, S3, and BB. A high ratio of biomass to lysis buffers will result in poor lysis conditions and subsequently low DNA yield and purity. For determination of cell density, calibration of each individual spectrophotometer is required to facilitate accurate conversion of $OD_{600}$ measurements into the number of cells per milliliter. This can be achieved by plating serial dilutions of a culture onto LB-agar plates in the absence of antibiotics. The counted colonies are used to calculate the number of cells per milliliter, which is then set in relation to the measured $OD_{600}$ values.

### Analytical gel analysis

The success of the plasmid purification procedure can be monitored on an analytical gel (see Figure 2, page 30). We recommend removing and saving an aliquot of the cleared lysate. If the plasmid DNA is of low yield or quality, the sample and eluate can be analyzed by agarose gel electrophoresis to determine at what stage of the purification the problem occurred (see Troubleshooting Guide, pages 24–26).

### Capacity of CompactPrep Mega and Giga Spin columns

The plasmid binding capacity is 2.5 mg for the CompactPrep Mega Spin column and 10 mg for the CompactPrep Giga Spin column. Actual yields will depend on number of cells, culture volume, plasmid copy number, size of insert, and host strain. For more details, visit our plasmid resource page at www.qiagen.com/goto/plasmidinfo and click on the link "General Considerations for Optimal Results".

### Vacuum manifolds

A vacuum manifold is required to draw the liquids through the CompactPrep Mega and Giga Spin columns.

### QIAvac 24 Plus

The QIAvac 24 Plus can be used for processing the CompactPrep Mega and Giga spin columns in combination with the QIAvac Holder. A waste disposal vessel allowing sufficient volume for the amount of preparations should be attached to the vacuum manifold (see Appendix A, page 27).

### QIAvac Holder

The QIAvac Holder is designed to stabilize the CompactPrep spin columns and extenders on the QIAvac 24 Plus. It prevents movement of the assembled columns and extenders, particularly when containing large volumes of liquid. The QIAvac Holder should be cleaned with water or laboratory detergent after use. Ethanol should not be used.

Depending on the number of preparations and thus the total liquid volume, different setups of the QIAvac 24 Plus can be used. The different assemblies are described in Appendix A, page 27.

### QIAvac 24 Plus without waste disposal vessel

This setup can be used if the total liquid volume is <400 ml. Setup of the QIAvac 24 Plus is described in the QIAvac 24 Plus Handbook.

### QIAvac 24 Plus in combination with a waste disposal vessel

The QIAvac 24 Plus can be used in combination with the Connecting System if the total liquid volume is <1.5 l. Setup of the QIAvac 24 Plus with the Connecting System is described in the QIAvac 24 Plus Handbook.

Alternatively, or if a higher liquid volume has to be processed, the QIAvac 24 Plus can be used in combination with vacuum compatible waste bottles of 10 liters or 20 liters. Setup of the QIAvac 24 Plus with larger waste containers is described in Appendix A, page 27.

### Other vacuum manifolds

Ensure that the waste volume can be handled. Insert the CompactPrep columns into the luer connectors and follow the manufacturer's instructions for use.

### Centrifugation

The drying and elution centrifugation steps should be performed at 5,000 x *g* in a centrifuge with a swing-out rotor and adapters for 50 ml tubes.

### Using LyseBlue reagent

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

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

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

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

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

Upon addition of neutralization buffer (Buffer S3), LyseBlue turns colorless. The presence of a homogeneous solution with no traces of blue indicates that SDS from the lysis buffer has been effectively precipitated.

# Protocol: Plasmid DNA Purification Using CompactPrep Plasmid Mega Kits

This protocol is designed for the preparation of up to 2.5 mg high- or low-copy plasmid DNA using the CompactPrep Plasmid Mega Kit with a maximum culture volume of 500 ml (LB culture medium) or a pellet wet weight of 1.5 g from fermentation cultures.

### Important points before starting

- 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 between –200 and –600 millibar (–150 and –450 mmHg). The vacuum pressure is measured as differential pressure between the inside of the bottle and the atmosphere (1013 millibar or 760 mmHg). Vacuum recommendations are given in negative units to indicate the required reduction in pressure with respect to the atmosphere.
- To avoid the possibility of implosion, do not use plastic/glass bottles or any other vessels that are not designed for use with a vacuum. Do not use plastic/glass bottles or any other vessels that are cracked or scratched. Wear safety glasses when working near a bottle under vacuum.
- Optional: Samples can be removed after step 6 of the protocol in order to monitor the procedure on an analytical gel (see Appendix B, page 30).

### Things to do before starting

- Add the provided RNase A solution to Buffer P1 before use. Use one complete vial of RNase A (spin down briefly before use) per bottle of Buffer P1, to give a final concentration of 100 µg/ml.
- Optional: Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use one complete vial of LyseBlue (centrifuge briefly before use) per bottle of Buffer P1 to achieve a 1:1000 dilution. Alternatively, add 1/1000 volume of LyseBlue reagent to an aliquot of Buffer P1 for the required number of samples and mix before use (for more details, see page 15).
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- Check Buffer P2 and Buffer BB for precipitation due to low storage temperature and, if necessary, dissolve by warming to 37°C.
- Close the bottle containing Buffer P2 immediately after use to avoid acidification of Buffer P2 from $CO_2$ in the air.

- Assemble the vacuum manifold with an appropriate waste container as described in Appendix A, page 27.

**Procedure**

1. **Screw the QIAfilter Mega-Giga cartridge onto a 45 mm-neck glass bottle and connect it to a vacuum source. Assemble the CompactPrep Mega columns with the tube extenders and position on the QIAvac 24 Plus as described in Appendix A, page 27.**

   Take care not to over-tighten the QIAfilter cartridge when attaching it to the neck of the bottle as this may cause the cartridge to crack.

2. **Resuspend pelleted bacteria in 25 ml Buffer P1.**

   Ensure that RNase A has been added to Buffer P1 (see "Things to do before starting".

   If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle before use to ensure LyseBlue particles are completely resuspended. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

   For efficient lysis, it is important to use a vessel large enough to allow complete mixing of the lysis buffers.

3. **Add 25 ml Buffer P2. Gently but thoroughly mix by inverting 4-6 times and incubate at room temperature (15–25°C) for 5 min.**

   The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min. After use, the bottle containing Buffer P2 should be closed immediately to avoid acidification from $CO_2$ in the air.

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

4. **Add 25 ml Buffer S3 to the lysate and mix immediately by inverting 4–6 times. Proceed directly to step 5. Do not incubate the lysate on ice.**

   After addition of Buffer S3, a fluffy white precipitate containing genomic DNA, proteins, cell debris, and potassium dodecyl sulfate (KDS) becomes visible. It is important to transfer the lysate into the QIAfilter cartridge immediately in order to prevent later disruption of the precipitate layer. If the mixture still appears viscous and brownish, more mixing is required to completely neutralize the solution.

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

5. **Transfer the lysate to the QIAfilter Mega-Giga cartridge and incubate at room temperature for 10 min.**

   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 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.

   **Note**: Incubation of the QIAfilter Mega-Giga cartridge at room temperature for 10 minutes is essential for optimal performance of the cartridge.

   Alternatively, the lysate can be centrifuged at 10,000 x *g* for 10 min using an appropriate vessel and centrifuge. After centrifugation, transfer the supernatant onto the QIAfilter Mega-Giga Cartridge. Do not incubate. Proceed to step 6.

6. **Switch on the vacuum source. After all liquid has been drawn through the QIAfilter cartridge, switch off the vacuum source.**
7. **Add 25 ml Buffer BB to the cleared lysate and mix by inverting 4–6 times.**
8. **Transfer the lysate to a CompactPrep column with a tube extender attached on the QIAvac 24 Plus.**
9. **Switch on the vacuum source, apply –300 mbar pressure and open the VacValves. After the liquid has been drawn through a column, close the respective VacValve. After the liquid has been drawn through all columns, switch off the vacuum source.**

   Closing VacValves after liquid has been drawn through the first columns ensures that the vacuum pressure remains constant and stable for the remaining samples.

10. **To wash the DNA, add 80 ml Buffer CPW, switch on the vacuum source, apply maximum vacuum and open the VacValves. After the liquid has been drawn through a column, close the respective VacValve. After the liquid has been drawn through all columns, switch off the vacuum source.**
11. **For the second wash step, add 50 ml Buffer PE, switch on the vacuum source, apply maximum vacuum, and open the VacValves. After the liquid has been drawn through a column, close the respective VacValve. After the liquid has been drawn through all columns, switch off the vacuum source.**
12. **Transfer the CompactPrep column into a 50 ml collection tube (supplied).**
13. **Centrifuge at 5000 x *g* for 10 min at room temperature to dry the membrane.**

14. **Place the CompactPrep column into a new 50 ml collection tube. To elute the DNA, add 1 ml Buffer EB to the CompactPrep column and let stand for 1 min. After incubation, close the tube and centrifuge at 5000 x *g* for 5 min at room temperature.**

    Water or buffers commonly used to dissolve DNA (e.g., TE) may also be used for elution.

    Note: TE buffer contains EDTA, which may inhibit downstream enzymatic or sequencing reactions.

    Note: Store DNA at – 20°C when eluted with water as DNA may degrade in the absence of buffering and chelating agents.

### Determination of yield

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

### Agarose gel analysis

We recommend removing and saving an aliquot of the cleared lysate (step 6). If the plasmid DNA is of low yield or quality, the sample and eluate can be analyzed by agarose gel electrophoresis to determine at what stage of the purification procedure the problem occurred (see Appendix B, page 30).

# Protocol: Plasmid DNA Purification Using CompactPrep Plasmid Giga Kits

This protocol is designed for the preparation of up to 10 mg high-copy plasmid DNA or of low-copy plasmid DNA using the CompactPrep Plasmid Giga Kit with a maximum culture volume of 2.5 liters (LB culture medium) or a pellet wet weight of 7.5 g from fermentation cultures.

### Important points before starting

- 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 between –200 and –600 millibar (–150 and –450 mmHg). The vacuum pressure is measured as differential pressure between the inside of the bottle and the atmosphere (1013 millibar or 760 mmHg). Vacuum recommendations are given in negative units to indicate the required reduction in pressure with respect to the atmosphere.
- To avoid the possibility of implosion, do not use plastic/glass bottles or any other vessels that are not designed for use with a vacuum. Do not use plastic/glass bottles or any other vessels that are cracked or scratched. Wear safety glasses when working near a bottle under vacuum.
- Optional: Samples can be removed after step 6 of the protocol in order to monitor the procedure on an analytical gel (see Appendix B, page 30).

### Things to do before starting

- Add the provided RNase A solution to Buffer P1 before use. Use one complete vial of RNase A (spin down briefly before use) per bottle of Buffer P1, to give a final concentration of 100 µg/ml.
- Optional: Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use one complete vial of LyseBlue (centrifuge briefly before use) per bottle of Buffer P1 to achieve a 1:1000 dilution. Alternatively, add 1/1000 volume of LyseBlue reagent to an aliquot of Buffer P1 for the required number of samples and mix before use (for more details, see page 15).
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- Check Buffer P2 and Buffer BB for precipitation due to low storage temperature and, if necessary, dissolve by warming to 37°C.
- Close the bottle containing Buffer P2 immediately after use to avoid acidification of Buffer P2 from $CO_2$ in the air.

- Assemble the vacuum manifold with an appropriate waste container as described in Appendix A, page 27.

**Procedure**

1. **Screw the QIAfilter Mega-Giga cartridge onto a 45 mm-neck glass bottle and connect it to a vacuum source. Assemble the CompactPrep Giga columns with the tube extenders and position on the QIAvac 24 Plus as described in Appendix A, page 27.**

   Take care not to over-tighten the QIAfilter cartridge when attaching it to the neck of the bottle as this may cause the cartridge to crack.

2. **Resuspend pelleted bacteria in 100 ml Buffer P1.**

   Ensure that RNase A has been added to Buffer P1 (see "Things to do before starting".

   If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle before use to ensure LyseBlue particles are completely resuspended. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

   For efficient lysis, it is important to use a vessel large enough to allow complete mixing of the lysis buffers.

3. **Add 100 ml Buffer P2. Gently but thoroughly mix by inverting 4-6 times and incubate at room temperature (15–25°C) for 5 min.**

   The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min. After use, the bottle containing Buffer P2 should be closed immediately to avoid acidification from $CO_2$ in the air.

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

4. **Add 100 ml Buffer S3 to the lysate and mix immediately by inverting 4–6 times. Proceed directly to step 5. Do not incubate the lysate on ice.**

   After addition of Buffer S3, a fluffy white precipitate containing genomic DNA, proteins, cell debris, and potassium dodecyl sulfate (KDS) becomes visible. It is important to transfer the lysate into the QIAfilter cartridge immediately in order to prevent later disruption of the precipitate layer. If the mixture still appears viscous and brownish, more mixing is required to completely neutralize the solution.

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

5. **Transfer the lysate to the QIAfilter Mega-Giga cartridge and incubate at room temperature for 10 min.**

   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 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.

   **Note**: Incubation of the QIAfilter Mega-Giga cartridge at room temperature for 10 minutes is essential for optimal performance of the cartridge.

   Alternatively, the lysate can be centrifuged at 10,000 x *g* for 10 min using an appropriate vessel and centrifuge. After centrifugation, transfer the supernatant onto the QIAfilter Mega-Giga Cartridge. Do not incubate. Proceed to step 6.

6. **Switch on the vacuum source. After all liquid has been drawn through the QIAfilter cartridge, switch off the vacuum source.**
7. **Add 100 ml Buffer BB to the cleared lysate and mix by inverting 4–6 times.**
8. **Transfer the lysate to a CompactPrep column with a tube extender attached on the QIAvac 24 Plus.**
9. **Switch on the vacuum source, apply -300 mbar pressure and open the VacValves. After the liquid has been drawn through a column, close the respective VacValve. After the liquid has been drawn through all columns, switch off the vacuum source.**

   Closing VacValves after liquid has been drawn through the first columns ensures that the vacuum pressure remains constant and stable for the remaining samples.

10. **To wash the DNA, add 80 ml Buffer CPW, switch on the vacuum source, apply maximum vacuum and open the VacValves. After the liquid has been drawn through a column, close the respective VacValve. After the liquid has been drawn through all columns, switch off the vacuum source.**
11. **For the second wash step, add 50 ml Buffer PE, switch on the vacuum source, apply maximum vacuum, and open the VacValves. After the liquid has been drawn through a column, close the respective VacValve. After the liquid has been drawn through all columns, switch off the vacuum source.**
12. **Transfer the CompactPrep column into a 50 ml collection tube (supplied).**
13. **Centrifuge at 5000 x *g* for 10 min at room temperature to dry the membrane.**

14. **Place the CompactPrep column into a new 50 ml collection tube. To elute the DNA, add 5 ml Buffer EB to the CompactPrep column and let stand for 1 min. After incubation, close the tube and centrifuge at 5000 x *g* for 5 min at room temperature.**

    Water or buffers commonly used to dissolve DNA (e.g., TE) may also be used for elution.

    Note: TE buffer contains EDTA, which may inhibit downstream enzymatic or sequencing reactions.

    Note: Store DNA at – 20°C when eluted with water as DNA may degrade in the absence of buffering and chelating agents.

### Determination of yield

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

### Agarose gel analysis

We recommend removing and saving an aliquot of the cleared lysate (step 6). If the plasmid DNA is of low yield or quality, the sample and eluate can be analyzed by agarose gel electrophoresis to determine at what stage of the purification procedure the problem occurred (see Appendix B, page 30).

# 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 CompactPrep column, culture volume, and culture media will ensure consistent and optimal results.

The following troubleshooting guide as well as the "General Considerations for Optimal Results" provided on our web page www.qiagen.com/goto/plasmidinfo may be helpful in solving any problems that may arise. The scientists at QIAGEN Technical Service are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com ).

| | Comments and suggestions |
|---|---|
| **Low or no yield** | |
| **No DNA in the cleared lysate before loading** | |
| a) Plasmid did not propagate | Check that the conditions for optimal growth were met. For more details, see www.qiagen.com/goto/plasmidinfo . |
| b) Cell resuspension incomplete | Pelleted cells should be completely resuspended in Buffer P1. Do not add Buffer P2 until an even suspension is obtained. |
| c) Alkaline lysis was inefficient | Insufficient mixing of lysis reagents will result in reduced yield. Mix thoroughly after addition of Buffers P1, P2, and S3 to achieve homogeneous suspensions. Use LyseBlue to visualize efficiency of mixing.<br><br>If cells have grown to very high densities or a larger amount of culture medium than recommended was used, the ratio of the biomass to lysis reagent is affected. This may result in poor lysis conditions as the volumes of Buffers P1, P2, and S3 are not sufficient to efficiently free plasmid DNA. Reduce the culture volume or, for CompactPrep Mega, increase volumes of Buffers P1, P2, S3, and BB in order to improve the ratio of biomass to lysis buffer. |

| | Comments and suggestions |
|---|---|
| d) Lysate prepared incorrectly | Check Buffer P2 for SDS precipitation resulting from low storage temperatures. Dissolve the SDS by warming.<br>The bottle containing Buffer P2 should always be closed immediately after use. |
| **DNA is found in the wash flow-through** | |
| Ethanol omitted from wash buffer | Repeat procedure with correctly prepared wash buffer (Buffer PE). |
| **Low DNA quality** | |
| Eluate contains residual ethanol | Ensure that the CompactPrep column is dried sufficiently. |
| **QIAfilter cartridge clogs during filtration** | |
| a) Culture volume too large | Do not exceed the culture volume recommended in the protocol. |
| b) Inefficient mixing after addition of Buffer S3 | Mix well until a fluffy white material has formed and the lysate is no longer viscous. |
| c) Mixing too vigorous after addition of Buffer S3 | After addition of Buffer S3, 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 S3 | After addition of Buffer S3, the lysate should be poured into the QIAfilter cartridge immediately. Decanting after incubation may disrupt the precipitate into tiny particles which may clog the QIAfilter cartridge. |
| e) QIAfilter cartridge was agitated during incubation | Pour the lysate into the QIAfilter cartridge immediately after addition of Buffer S3 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 S3 on ice instead of room temperature | Ensure incubation is performed at room temperature (15–25°C) in the QIAfilter Cartridge. Precipitate flotation is more efficient at room temperature than on ice. |

| | Comments and suggestions |
|---|---|
| g) Incubation time after addition of Buffer S3 too short | Incubate with Buffer S3 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 around the cartridge wall to dislodge the precipitate before continuing with the filtration. |
| h) Vacuum pressure was too weak | Ensure that the vacuum generates a vacuum pressure of –200 to –600 millibar (–150 to –450 mmHg). |

# Appendix A: Setup of the QIAvac 24 Plus

For more information about the QIAvac 24 Plus and cleaning instructions, please read the *QIAvac 24 Plus Handbook* (available from www.qiagen.com ).

Up to 12 CompactPrep Mega or Giga preps can be processed in parallel on the QIAvac 24 Plus. Depending on the number of plasmid preparations, different waste volumes are generated. Tables 3 and 4 may help to determine an appropriate waste container.

**Table 3. Waste volumes**

| | CompactPrep Plasmid Mega Kit | | CompactPrep Plasmid Giga Kit | |
|---|---|---|---|---|
| | **1 prep** | **12 preps** | **1 prep** | **12 preps** |
| Lysate | 100 ml | 1.2 liters | 400 ml | 4.8 liters |
| Wash buffers | 130 ml | 1.6 liters | 130 ml | 1.6 liters |
| Complete preparation | 230 ml | 2.8 liters | 530 ml | 6.4 liters |

**Table 4. Volume capacity**

| Setup | Assembly and waste container | Volume capacity |
|---|---|---|
| A | QIAvac 24 Plus and QIAvac Connecting System | 1.5 liters |
| B | QIAvac 24 Plus without waste container | 400 ml |
| C | QIAvac 24 Plus and 10 liter or 20 liter waste container | 8 liters or 18 liters |

**Procedure**

We recommend the use of a vacuum regulator in order to monitor and regulate the vacuum pressure. For more details on the vacuum regulator, please refer to the *QIAvac 24 Plus Handbook*.

1. **[A] If the QIAvac 24 Plus is used together with the QIAvac Connecting System, assemble the system as described in the *QIAvac 24 Plus Handbook* on page 16–20.**

   **[B] If the QIAvac 24 Plus is used as a stand alone module, assemble as described in the *QIAvac 24 Plus Handbook* on page 15.**

   **[C] If 10 liter or 20 liter waste containers are used, assemble the QIAvac 24 Plus as described in the *QIAvac 24 Plus Handbook* on page 16. Connect the waste container with the QIAvac 24 Plus. Connect the waste container to the vacuum pump using the tubing with a vacuum regulator.**

The following components may be used:

- 10 liter waste container (e.g., Nalgene Heavy Duty Vacuum Carboy [PP], cat. no. 2226-0020)
- 20 liter waste container (e.g., Nalgene Heavy Duty Vacuum Carboy [PP], cat. no. 2226-0050)
- Quick filling/venting closure (e.g., Nalgene, cat. no. 2158-0021)
- Vacuum tubing (e.g., Nalgene 180 clear plastic vacuum tubing, cat. no. 8000-0065 [50 ft. per case])
- Vacuum tubing (e.g., Nalgene 180 clear plastic vacuum tubing, cat. no. 8000-0065 [10 ft. per case])
- Vacuum regulator (e.g., QIAGEN cat. no. 19530)

2. **Place the QIAvac 24 Plus into the QIAvac Holder on a flat surface.**
3. **Insert the required number of VacValves into every second luer slot of the QIAvac 24 Plus.**

   Make sure that the VacValves are inserted into the luer slots below the hole in the QIAvac Holder.

4. **Place the required number of CompactPrep columns pre-assembled with extenders into the VacValves.**

   Optional: VacConnectors can be used to avoid direct contact of CompactPrep columns with the VacValves. Insert a VacConnector into each VacValve and place the CompactPrep columns into the VacConnectors.

   For a schematic drawing of the assembly, see Figure 1.

5. **Close unused luer slots of the manifold with luer caps.**
6. **Adjust the vacuum pressure to –300 mbar: Close the main vacuum valve on the vacuum regulator and the VacValves on the QIAvac 24 Plus. Switch on the vacuum pump. Wait for the needle of the vacuum regulator to stabilize. Adjust vacuum to –300 mbar using the vacuum valve on the vacuum regulator.**
7. **Carefully open the main vacuum valve and switch off the vacuum pump.**

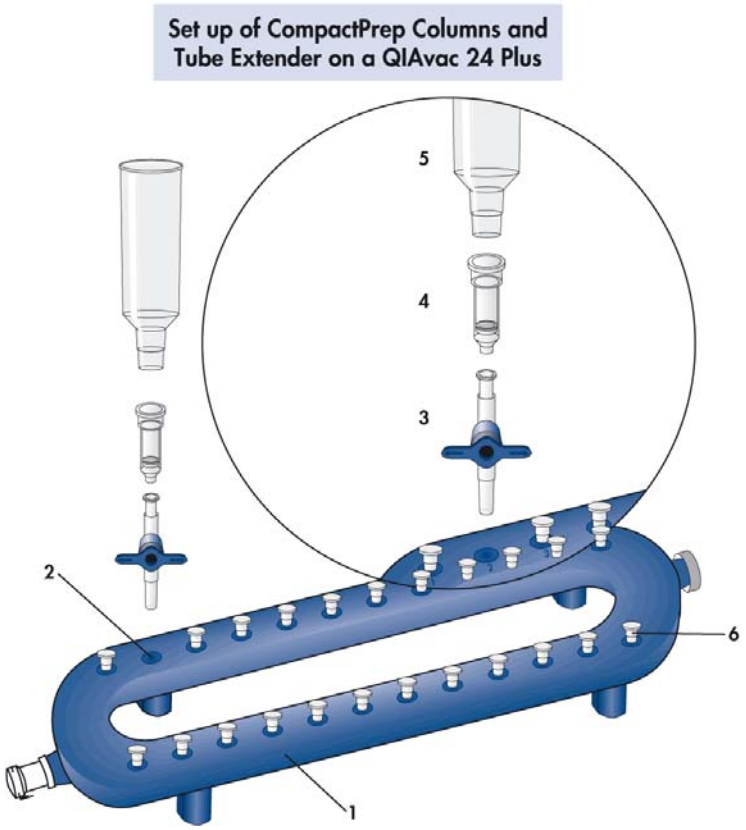



**Figure 1. Setting up the QIAvac 24 Plus with CompactPrep columns using VacValves**

1. QIAvac 24 Plus vacuum manifold
2. Luer slot of the QIAvac 24 Plus
3. VacValve*
4. CompactPrep column
5. Tube extender
6. Luer slot closed with luer plug

* Must be purchased separately.

# Appendix B: 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 a fraction of the cleared lysate and analyze by agarose gel electrophoresis.

## Preparation of samples

Remove an aliquot from the cleared lysate as indicated in the protocol. Precipitate the nucleic acids by adding 1 volume of isopropanol*, centrifuge for 15 min at maximum speed, and discard supernatant. Rinse the plasmid DNA pellets with 70% ethanol, drain well, and resuspend in 10 µl TE, pH 8.0.

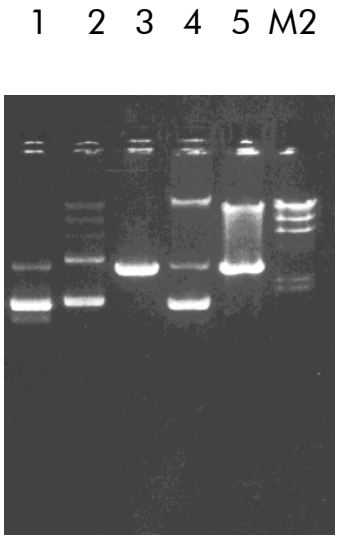



**Figure 2. Agarose gel analysis of the plasmid purification procedure.**

## Agarose gel analysis

Run 2 µl of cleared lysate sample on a 1% agarose gel and compare to the eluted plasmid DNA as shown in Figure 2. 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 24–26. If the problem remains unresolved, or if you have any further questions, please call QIAGEN Technical Service.

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

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 will be 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.

**M2**: Lambda DNA digested with *Hin*dIII.

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| CompactPrep Plasmid Mega Kit (5)* | 5 CompactPrep Mega-Giga Columns, Extender Tubes, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges | 12781 |
| CompactPrep Plasmid Giga Kit (5)* | 5 CompactPrep Mega-Giga Columns, Extender Tubes, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges | 12791 |
| **Related products** | | |
| CompactPrep Plasmid Midi Kit (25)* | 25 CompactPrep Midi Columns, Extender Tubes, Reagents, Buffers, 25 QIAfilter Midi Cartridges | 12743 |
| CompactPrep Plasmid Midi Kit (100)* | 100 CompactPrep Midi Columns, Extender Tubes, Reagents, Buffers, 100 QIAfilter Midi Cartridges | 12745 |
| CompactPrep Plasmid Maxi Kit (25)* | 25 CompactPrep Maxi Columns, Extender Tubes, Reagents, Buffers, 25 QIAfilter Maxi Cartridges | 12763 |
| CompactPrep Plasmid Maxi Kit (100)* | 100 CompactPrep Maxi Columns, Extender tubes, Reagents, Buffers, 100 QIAfilter Maxi Cartridges | 12765 |
| CompactPrep Plasmid Midi Core Kit (25)* | 25 CompactPrep Midi Columns, Extender tubes, Reagents, Buffers | 12843 |
| CompactPrep Plasmid Midi Core Kit (100)* | 100 CompactPrep Midi Columns, Extender tubes, Reagents, Buffers | 12845 |
| CompactPrep Plasmid Maxi Core Kit (25)* | 25 CompactPrep Maxi Columns, Extender tubes, Reagents, Buffers | 12863 |
| CompactPrep Plasmid Maxi Core Kit (100)* | 100 CompactPrep Maxi Columns, Extender tubes, Reagents, Buffers | 12865 |

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

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| **Accessories** | | |
| QIAvac 24 Plus | Vacuum manifold for processing 1–24 spin columns: QIAvac 24 Plus Vacuum manifold, Luer Plugs, Quick Couplings | 19413 |
| QIAvac Holder | Stand for QIAvac 24 plus for parallel processing of up to 12 CompactPrep Mega/Giga columns | 19418 |
| QIAvac Connecting System | System to connect vacuum manifold with vacuum pump: includes Tray, Waste Bottles, Tubings, Couplings, Valve, Gauge, 24 VacValves | 19419 |
| VacValves (24) | 24 valves for use with the QIAvac 24 Plus | 19408 |
| VacConnectors (500) | 500 disposable connectors for optional use with CompactPrep Columns | 19407 |
| Vacuum Pump (110 V, 60 HZ) | Universal vacuum pump | 84000 |
| Vacuum Pump (115 V, 60 HZ) | Universal vacuum pump | 84010 |
| Vacuum Pump (230 V, 50 HZ) | Universal vacuum pump | 84020 |

## Notes

**www.qiagen.com**

**Australia** ■ Orders 03-9840-9800 ■ Fax 03-9840-9888 ■ Technical 1-800-243-066

**Austria** ■ Orders 0800/28-10-10 ■ Fax 0800/28-10-19 ■ Technical 0800/28-10-11

**Belgium** ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

**Canada** ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

**China** ■ Orders 021-51345678 ■ Fax 021-51342500 ■ Technical 021-51345678

**Denmark** ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

**Finland** ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

**France** ■ Orders 01-60-920-926 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930 ■ Offers 01-60-920-928

**Germany** ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

**Hong Kong** ■ Orders 800 933 965 ■ Fax 800 930 439 ■ Technical 800 930 425

**Ireland** ■ Orders 1800-555-049 ■ Fax 1800-555-048 ■ Technical 1800-555-061

**Italy** ■ Orders 02-33430411 ■ Fax 02-33430426 ■ Technical 800-787980

**Japan** ■ Telephone 03-5547-0811 ■ Fax 03-5547-0818 ■ Technical 03-5547-0811

**Luxembourg** ■ Orders 8002-2076 ■ Fax 8002-2073 ■ Technical 8002-2067

**The Netherlands** ■ Orders 0800-0229592 ■ Fax 0800-0229593 ■ Technical 0800-0229602

**Norway** ■ Orders 800-18859 ■ Fax 800-18817 ■ Technical 800-18712

**South Korea** ■ Orders 1544 7145 ■ Fax 1544 7146 ■ Technical 1544 7145

**Sweden** ■ Orders 020-790282 ■ Fax 020-790582 ■ Technical 020-798328

**Switzerland** ■ Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

**UK** ■ Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

**USA** ■ Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)


1050344 01/2008

Sample & Assay Technologies