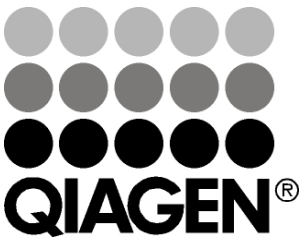
April 2012

QIAGEN® Plasmid *Plus* Purification Handbook

For preparation of transfection-grade plasmid DNA from *E. coli*



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

QIAGEN Plasmid *Plus* Kit	**Midi (25)**	**Midi (100)**
Catalog. no.	**12943**	**12945**
Number of preps.	**25**	**100**
QIAGEN Plasmid *Plus* Spin Columns	25 Midi columns	100 Midi columns
QIAfilter Midi Cartridges	25	100
Caps for QIAfilter	25	100
Tube Extenders (20 ml)	25	100
Collection Tubes (2 ml)	25	100
Buffer P1	110 ml	500 ml
Buffer P2	110 ml	500 ml
Buffer S3	2 x 70 ml	1 x 280 ml 2 x 70 ml
Buffer ETR	25 ml	3 x 25 ml
Buffer BB	70 ml	4 x 70 ml
Buffer PE (concentrate)	6 ml	2 x 10 ml
Buffer EB	15 ml	2 x 15 ml
RNase A*	11 mg	50 mg
LyseBlue®	110 µl	500 µl
Quick-Start Protocol	1	1

* Provided as a 10 mg/ml or 100 mg/ml solution.

QIAGEN Plasmid *Plus* Kit	**Maxi (25)**	**Maxi (100)**
Catalog. no.	**12963**	**12965**
Number of preps.	**25**	**100**
QIAGEN Plasmid *Plus* Spin Columns	25 Maxi columns	100 Maxi columns
QIAfilter Maxi Cartridges	25	100
Caps for QIAfilter	25	100
Tube Extenders (20 ml)	25	100
Collection Tubes (2 ml)	25	100
Buffer P1	250 ml	1 x 110 ml 3 x 250 ml
Buffer P2	250 ml	1 x 110 ml 3 x 250 ml
Buffer S3	3 x 70 ml	3 x 280 ml
Buffer ETR	25 ml	3 x 25 ml
Buffer BB	2 x 70 ml	4 x 160 ml
Buffer PE (concentrate)	6 ml	2 x 10 ml
Buffer EB	15 ml	55 ml
RNase A*	25 mg	1 x 11 mg 3 x 25 mg
LyseBlue	250 µl	110 µl 3 x 250 µl
Quick-Start Protocol	1	1

* Provided as a 10 mg/ml or 100 mg/ml solution.

QIAGEN Plasmid *Plus* Kit	**Mega (5)**	**Giga (5)**
Catalog no.	**12981**	**12991**
Number of preps	**5**	**5**
QIAGEN Plasmid *Plus* 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	150 ml	4 x 150 ml
Buffer P2	150 ml	4 x 150 ml
Buffer S3	2 x 70 ml	2 x 280 ml
Buffer BB	4 x 70 ml	4 x 160 ml
Buffer ETR	490 ml	490 ml
Buffer PE (concentrate)	2 x 55 ml	2 x 55 ml
Buffer EB	55 ml	2 x 55 ml
RNase A (100 mg/ml)	15 mg	4 x 15 mg
LyseBlue	150 µl	4 x 150 µl
Quick-Start Protocol	1	1

Storage

QIAGEN Plasmid *Plus* Kits should be stored dry at room temperature (15–25°C). Kits can be stored for up to 24 months without showing 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 and RNase A stock solution can be stored for 2 years at room temperature.

Intended Use

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

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

Safety Information

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

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

Quality Control

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

Introduction

QIAGEN Plasmid *Plus* Kits provide a novel method for ultrafast and easy large-scale plasmid preparation. The procedure can be performed in 20 (Midi and Maxi), 40 (Mega), or 50 minutes (Giga) using a vacuum pump and centrifuge. The design and unique binding chemistry of the QIAGEN Plasmid *Plus* columns allow processing of up to 12 (Mega and Giga) or 24 (Midi and Maxi) preparations in parallel on the QIAvac 24 Plus with an appropriate waste container.

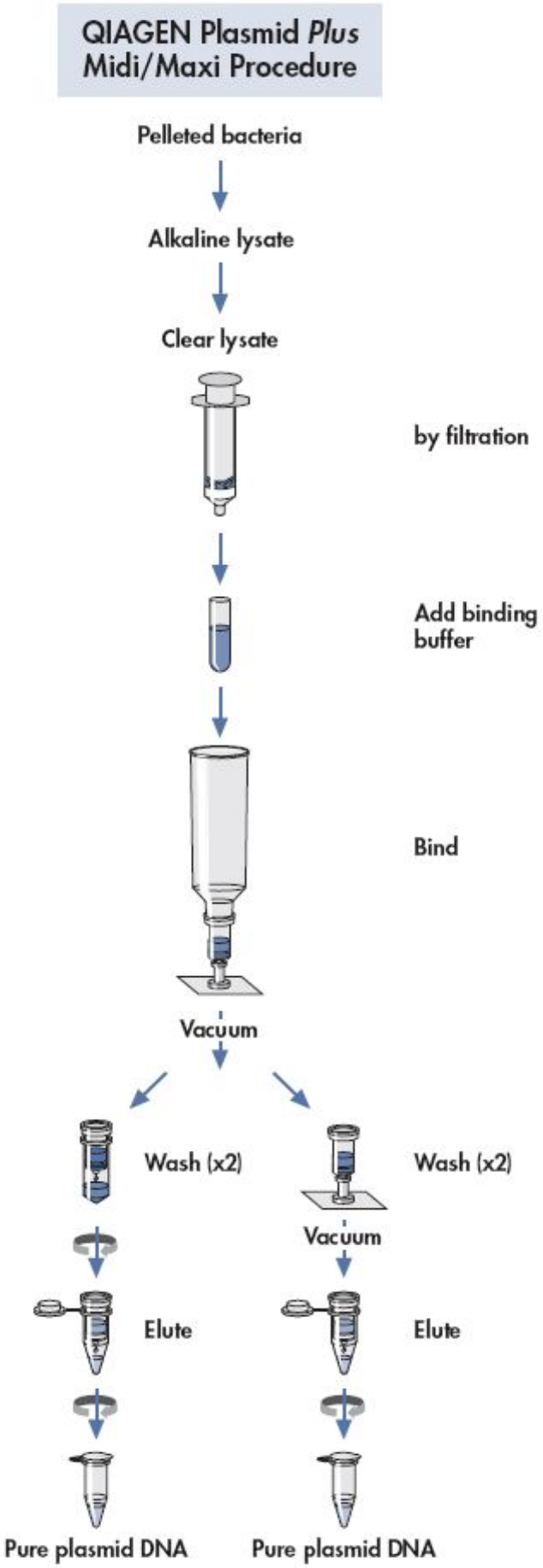
The procedure is based on a novel, proprietary chemistry (patent pending). After lysate clearing, the process follows a simple bind-wash-elute procedure. The resulting highly concentrated DNA is immediately ready for use in subsequent applications.

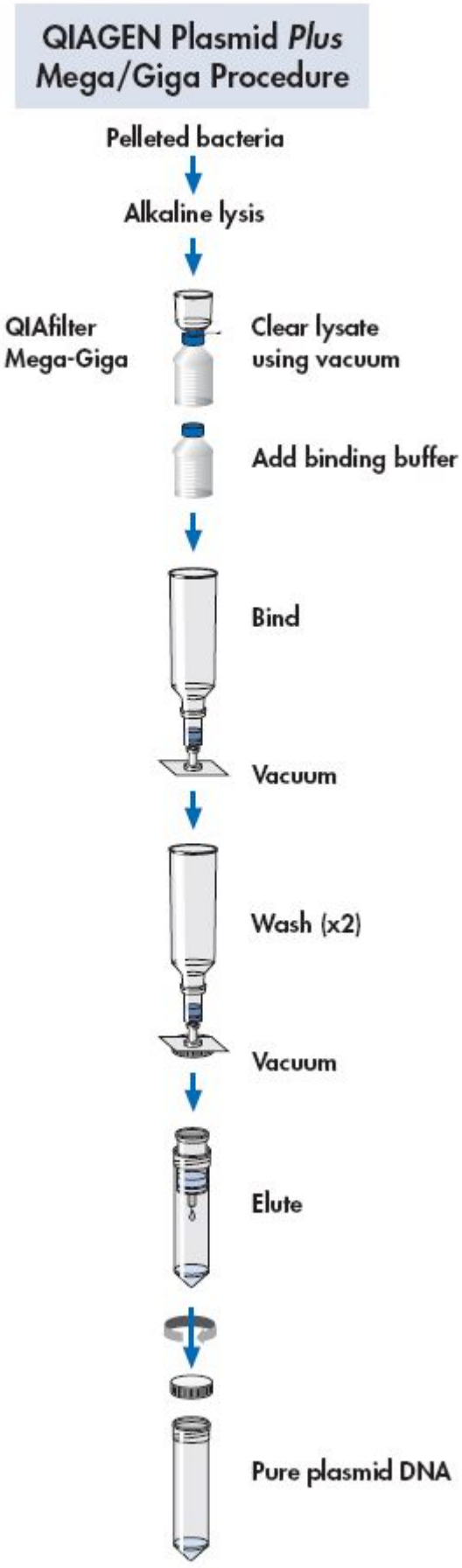
QIAGEN Plasmid *Plus* Kits provide transfection-grade DNA, highly suited for routine applications such as enzymatic modification, cloning, and sequencing, and for transfection into most cell lines (including sensitive cell lines such as Huh-7). Endo-free DNA quality may be required for transfection into highly sensitive cells. QIAGEN's EndoFree® Plasmid Kits offer the highest possible DNA quality and are highly suitable for a variety of 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.

LyseBlue reagent

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


QIAGEN Plasmid *Plus* Midi/Maxi Procedure
Pelleted bacteria
Alkaline lysate
Clear lysate
by filtration
Add binding buffer
Bind
Vacuum
Wash (x2)
Wash (x2)
Vacuum
Elute
Elute
Pure plasmid DNA
Pure plasmid DNA


QIAGEN Plasmid *Plus* 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.

For all protocols

- Standard microbiological equipment for growing and harvesting bacteria (e.g., inoculating loop, culture tubes and flasks, 37°C shaking incubator, and centrifuge with rotor and tubes or bottles for harvesting cells)
- 96–100% ethanol
- Microcentrifuge
- Vacuum manifold (e.g., QIAvac 24 Plus, cat. no. 19413)
- For the mega and giga procedure
- Centrifuge with swing-out rotor and adapters for 50 ml tubes
- VacValves (cat. no. 19408)
- QIAvac Holder (cat. no. 19418)
- For parallel processing of more than one QIAGEN Plasmid *Plus* Mega preparation or for QIAGEN Plasmid *Plus* Giga preparations on the QIAvac 24 Plus: QIAvac Connecting system (cat. no. 19419) or 10 liter or 20 liter vacuum bottles with tubing (see Appendix A, page 33)
- Vacuum pump (e.g., 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

Plasmid 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, DH5®α, and C600 yield high-quality DNA with QIAGEN protocols. The slower growing strain XL1-Blue also yields DNA of very high quality.

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

If the quality of purified DNA is not as expected, a change of host strain should be considered. If difficulty is encountered with strains such as TG1 and Top10F, we recommend reducing the amount of culture volume 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 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 15) to obtain the highest plasmid yields.

In general, we do not recommend using rich media with our plasmid kits. Using rich media might lead to clogging of the membrane and slower processing of the preparation. Overloading the membrane might even result in low yields. If this is the case, growth time and/or lysis conditions must be optimized, and culture volumes reduced. For more details, visit our plasmid resource page at

www.qiagen.com/goto/plasmidinfo and click on the link "General Considerations for Optimal Results".

Table 2. Composition of Luria Bertani medium

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

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 QIAGEN Plasmid *Plus* Kits is optimized for use with cultures grown in Luria Bertani (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, reduce the culture volumes accordingly. 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.

QIAGEN also offers an optional "high-yield protocol", which allows input of higher culture volumes, for use with QIAGEN Plasmid *Plus* Kits. Therefore, volumes of lysis buffers also have to be adapted. Please note that especially when using this protocol, the cell density needs to be monitored as described before. If the cell density is too high, the column can get clogged, leading to lower yields. Also, the process might be slowed down if the lysate is too viscous. In this case, the cell density/culture volumes have to be adapted.

Analytical gel analysis

The success of the plasmid purification procedure can be monitored on an analytical gel. 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 the stage of the purification where the problem occurred.

Using LyseBlue reagent

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

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

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

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

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

Upon addition of the 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.

Vacuum manifolds

Use of a vacuum manifold is required to draw the DNA solution into the QIAGEN Plasmid *Plus* spin column.

QIAvac 24 Plus

The QIAvac 24 Plus can be used for processing the QIAGEN Plasmid *Plus* spin columns. For Mega and Giga preps, it should be combined 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 33).

QIAvac Holder

The QIAvac Holder is designed to stabilize the QIAGEN Plasmid *Plus* Mega and Giga 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 33.

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 liter. 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 33.

Other vacuum manifolds

Ensure that the waste volume can be handled. Insert the QIAGEN Plasmid *Plus* spin columns into the luer connectors and follow the manufacturer's instructions for use.

Centrifugation

- For midi and maxi formats, all microcentrifugation steps are carried out at 10,000 x *g* (approximately 13,000 rpm) in a conventional tabletop microcentrifuge.
- For mega and giga formats, 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.

Things to do before starting for all protocols

- Add the provided RNase A solution to Buffer P1 before use. Use 1 vial RNase A (centrifuge briefly before use) per bottle Buffer P1 for a final concentration of 100 µg/ml.
- **Optional**: Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use 1 vial LyseBlue reagent per bottle Buffer P1 for a final dilution of 1:1000 (e.g., 10 µl LyseBlue into 10 ml Buffer P1). 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 16).
- 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 33.

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.

Protocol: Plasmid DNA Purification using QIAGEN Plasmid *Plus* Midi Kits

This protocol is designed for the preparation of up to 250 µg high-copy plasmid DNA or of low-copy plasmid DNA using the QIAGEN Plasmid *Plus* Midi Kit with a maximum culture volume of 35 ml.

Important points before starting

- Text marked with a ▲ denotes values for the QIAGEN Plasmid *Plus* Midi standard protocol; text marked with a ● denotes values for the QIAGEN Plasmid *Plus* Midi high-yield protocol.
- **Optional**: samples can be removed after step 6 of the protocol to monitor the procedure on an analytical gel.

Table 3. Maximum recommended culture volumes

Copy number	Standard protocol	High-yield protocol
High-copy plasmid*	20–25 ml	25–35 ml
Low-copy plasmid*†	50 ml	–

* For high-copy plasmids, expected yields are ▲100–200 µg for the QIAGEN Plasmid *Plus* Midi Kit using the standard protocol and ●150–250 µg for the QIAGEN Plasmid *Plus* Midi using the high-yield protocol. For low-copy plasmids, expected yields are ▲30–100 µg for the QIAGEN Plasmid *Plus* Midi Kit using these culture values with the standard protocol.

† Low-copy plasmids can be efficiently purified using the QIAGEN Plasmid *Plus* Midi Kit; however, growing bacteria to a high cell density or in rich media (e.g., Terrific-Broth [TB] or 2x YT) may lead to a reduction in plasmid purity. This is due to the increased ratio of contaminants (e.g., RNA, proteins, and polysaccharides) compared to plasmid DNA.

Procedure

1. **Resuspend pelleted bacteria in ▲ 2 ml or ● 4 ml Buffer P1.**

   Ensure that RNase A has been added to Buffer P1 (see "Things to do before starting" on page 18 for all protocols).

   For efficient lysis, it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

2. **Add ▲ 2 ml or ● 4 ml Buffer P2, gently mix by inverting, and incubate at room temperature (15–25°C) for 3 min.**

   The lysate should appear viscous. Do not allow lysis 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.

3. **Add ▲ 2 ml or ● 4 ml Buffer S3 to the lysate and mix immediately by inverting 4–6 times. Proceed directly to step 4. Do not incubate the lysate on ice.**

   After addition of Buffer S3, 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 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 SDS has been effectively precipitated.

4. **Transfer the lysate to the QIAfilter cartridge and incubate at room temperature for 10 min.**

   If working with low-copy plasmid culture volumes, it may be beneficial to centrifuge the lysate before clarifying through QIAfilter cartridge. The centrifugation is performed instead of the 10 min incubation and leads to convenient filtration without clogging. The centrifugation can be performed directly in the same vessel which is used for alkaline lysis if this vessel is appropriate for centrifugation. Centrifugation should be performed for 5 min at approx. 4500 x *g*. Minor amounts of the precipitated material will be on the surface of the supernatant and can easily be removed with a pipet tip while pouring the supernatant into the QIAfilter cartridge.

   Important: Room-temperature incubation is essential for optimal performance of the QIAfilter 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 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.

5. **During incubation, prepare the vacuum manifold and the QIAGEN Plasmid *Plus* Midi spin columns.**

6. **Gently insert the plunger into the QIAfilter cartridge and filter the cell lysate into a new tube, allowing space for the addition of Buffer BB.**

   Filter until all of the lysate has passed through the QIAfilter cartridge, but do not apply extreme force.

7. **Add 2 ml Buffer BB to the cleared lysate and mix by inverting 4–6 times.**
8. **Transfer lysate to a QIAGEN Plasmid *Plus* Midi spin column with a tube extender attached on the QIAvac 24 Plus.**
9. **Switch on the vacuum source, apply approximately –300 mbar, and draw the solution through the QIAGEN Plasmid *Plus* Midi spin column. After the liquid has been drawn through all columns, switch off the vacuum source.**

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

10. **To wash the DNA, add 0.7 ml Buffer ETR and proceed with one of the following two steps:**

    Washing using a vacuum:

- After adding 0.7 ml Buffer ETR, switch on the vacuum source. After the liquid has been drawn through all columns, switch off the vacuum source.

Washing using a microcentrifuge:

- Discard the tube extenders and place the QIAGEN Plasmid *Plus* Midi spin column into the 2 ml collection tube provided. Wash the column by adding 0.7 ml Buffer ETR and centrifuging for 1 min at 10,000 x *g*. Discard the flow-through.

11. **To further wash the DNA, add 0.7 ml Buffer PE and proceed with one of the following two steps:**

    Washing using a vacuum:

- After adding 0.7 ml Buffer PE, switch on the vacuum source. After the liquid has been drawn through all columns, switch off the vacuum source.

Washing using a microcentrifuge:

- Place the QIAGEN Plasmid *Plus* Midi spin column in the empty collection tube from step 12. Wash the DNA by adding 0.7 ml Buffer PE and centrifuging for 1 min at 10,000 x *g*. Discard the flow-through.

12. **To completely remove the residual wash buffer, centrifuge the column for 1 min at 10,000 x *g* in a microcentrifuge.**

13. Place the QIAGEN Plasmid *Plus* Midi spin column into a clean 1.5 ml microcentrifuge tube. To elute the DNA, add 200 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the QIAGEN Plasmid *Plus* Midi spin column, let it stand for at least 1 min, and centrifuge for 1 min.

Water or buffers (e.g., TE buffer) commonly used to dissolve DNA 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.

Protocol: Plasmid DNA Purification using QIAGEN Plasmid *Plus* Maxi Kits

This protocol is designed for the preparation of up to 1000 µg high-copy plasmid DNA or of low-copy plasmid DNA using the QIAGEN Plasmid *Plus* Maxi Kit with a maximum culture volume of 130 ml.

Important points before starting

- Text marked with a ▲ denotes values for the QIAGEN Plasmid *Plus* Maxi standard protocol; text marked with a ● denotes values for the QIAGEN Plasmid *Plus* Maxi high-yield protocol.
- **Optional:** samples can be removed after step 6 of the protocol to monitor the procedure on an analytical gel.

Table 4. Maximum recommended culture volumes

Copy number	Standard protocol	High-yield protocol
High-copy plasmid*	80–100 ml	100–130ml
Low-copy plasmid*†	up to 200 ml	–

* For high-copy plasmids, expected yields are ▲ up to 750 µg for the QIAGEN Plasmid *Plus* Maxi Kit using the standard protocol and ● up to 1000 µg for the QIAGEN Plasmid *Plus* Maxi using the high-yield protocol. For low-copy plasmids, expected yields are ▲50–250 µg for the QIAGEN Plasmid *Plus* Maxi Kit using the standard protocol with culture volumes of up to 200 ml.

† Low-copy plasmids can be efficiently purified using the QIAGEN Plasmid *Plus* Maxi Kit; however, growing bacteria to a high cell density or in rich media (e.g., Terrific-Broth [TB] or 2x YT) may lead to a reduction in plasmid purity. This is due to the increased ratio of contaminants (e.g., RNA, proteins, and polysaccharides) compared to plasmid DNA.

Procedure

1. **Resuspend pelleted bacteria in ▲ 5 ml or ● 8 ml Buffer P1.**

   Ensure that RNase A has been added to Buffer P1 (see "Things to do before starting" on page 18 for all protocols).

   For efficient lysis, it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

2. **Add ▲ 5 ml or ● 8 ml Buffer P2, gently mix by inverting, and incubate at room temperature (15–25°C) for 3 min.**

   The lysate should appear viscous. Do not allow lysis 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.

3. **Add ▲ 5 ml or ● 8 ml Buffer S3 to the lysate and mix immediately by inverting 4–6 times. Proceed directly to step 4. Do not incubate the lysate on ice.**

   After addition of Buffer S3, 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 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 SDS has been effectively precipitated.

4. **Transfer the lysate to the QIAfilter cartridge and incubate at room temperature for 10 min.**

   If working with low-copy plasmid culture volumes, it may be beneficial to centrifuge the lysate before clarifying through QIAfilter cartridge. The centrifugation is performed instead of the 10 minute incubation and leads to convenient filtration without clogging. The centrifugation can be performed directly in the same vessel that is used for alkaline lysis if this vessel is appropriate for centrifugation. Centrifugation should be performed for 5 min at approx. 4500 x *g*. Minor amounts of the precipitated material will be on the surface of the supernatant and can easily be removed with a pipet tip while pouring the supernatant into the QIAfilter cartridge.

   Important: Room-temperature incubation is essential for optimal performance of the QIAfilter 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 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.

5. **During incubation, prepare the vacuum manifold and QIAGEN Plasmid *Plus* Maxi spin columns.**

6. **Gently insert the plunger into the QIAfilter cartridge and filter the cell lysate into a new tube, allowing space for the addition of Buffer BB.**

   Filter until all of the lysate has passed through the QIAfilter cartridge, but do not apply extreme force.

7. **Add 5 ml Buffer BB to cleared lysate and mix by inverting 4–6 times.**
8. **Transfer lysate to a QIAGEN Plasmid *Plus* Maxi spin column with a tube extender attached on the QIAvac 24 Plus.**
9. **Switch on the vacuum source, apply approximately –300 mbar, and draw the solution through the QIAGEN Plasmid *Plus* Maxi spin column. After the liquid has been drawn through all columns, switch off the vacuum source.**

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

10. **To wash the DNA, add 0.7 ml Buffer ETR and proceed with one of the following two steps:**

    Washing using a vacuum:

- After adding 0.7 ml Buffer ETR, switch on the vacuum source. After the liquid has been drawn through all columns switch off the vacuum source.

Washing using a microcentrifuge:

- Discard the tube extenders and place the QIAGEN Plasmid *Plus* Maxi spin column into the 2 ml collection tube provided. Wash the column by adding 0.7 ml Buffer ETR and centrifuging for 1 min at 10,000 x *g*. Discard the flow-through.

11. **To further wash the DNA, add 0.7 ml Buffer PE and proceed with one of the following two steps:**

    Washing using a vacuum:

- After adding 0.7 ml Buffer PE, switch on the vacuum source. After the liquid has been drawn through all columns, switch off the vacuum source.

Washing using a microcentrifuge:

- Place the QIAGEN Plasmid *Plus* Maxi spin column in the empty collection tube from step 12. Wash the DNA by adding 0.7 ml Buffer PE and centrifuging for 1 min at 10,000 x *g*. Discard the flow-through.

12. **To completely remove the residual wash buffer, centrifuge the column for 1 min at 10,000 x *g* in a microcentrifuge.**

13. **Place the QIAGEN Plasmid *Plus* Maxi spin column into a clean 2 ml microcentrifuge tube. To elute the DNA, add 400 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the QIAGEN Plasmid *Plus* Maxi spin column, let it stand for at least 1 min, and centrifuge for 1 min.**

    Water or buffers (e.g., TE) commonly used to dissolve DNA 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.

Protocol: Plasmid DNA Purification using QIAGEN Plasmid *Plus* Mega or Giga Kits

This protocol is to be followed when using QIAGEN Plasmid *Plus* Mega or Giga Kits. When using a maximum culture volume of 500 ml (LB culture medium) or a pellet wet weight of 1.5 g from fermentation cultures, up to 2.5 mg high- or low-copy plasmid DNA can be prepared using the QIAGEN Plasmid *Plus* Mega Kit. When using a maximum culture volume of 2.5 liters (LB culture medium) or a pellet wet weight of 7.5 g from fermentation cultures, up to 10 mg high- or low-copy plasmid DNA can be prepared using the QIAGEN Plasmid *Plus* Giga Kit.

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 7 of the protocol to monitor the procedure on an analytical gel.
- Text marked with a ▲ denotes values for the QIAGEN Plasmid *Plus* Mega Kit; text marked with a ● denotes values for the QIAGEN Plasmid *Plus* Giga Kit.

Procedure

1. **Screw the QIAfilter Mega-Giga cartridge onto a 45 mm-neck glass bottle and connect it to a vacuum source. Assemble the QIAGEN Plasmid *Plus* Mega or Giga spin columns with the tube extenders and position on the QIAvac 24 Plus as described in Appendix A, page 33.**

   Take care not to overtighten 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 or ● 100 ml Buffer P1.**

   Ensure that RNase A has been added to Buffer P1 (see "Things to do before starting" on page 18).

   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.

3. **Add ▲ 25 ml or ● 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 lysis 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 or ● 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 SDS becomes visible. It is important to transfer the lysate into the QIAfilter cartridge immediately 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 SDS 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 or ● 100 ml Buffer BB to the cleared lysate and mix by inverting 4–6 times.**
8. **Transfer the lysate to a QIAGEN Plasmid *Plus* Mega or Giga spin 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 ETR, 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 QIAGEN Plasmid *Plus* Mega or Giga spin 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 QIAGEN Plasmid *Plus* Mega or Giga spin column into a new 50 ml collection tube. To elute the DNA, add ▲1 ml or ● 5 ml Buffer EB to the QIAGEN Plasmid *Plus* Mega or Giga spin 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 (e.g., TE) commonly used to dissolve DNA 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.

Troubleshooting Guide

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

	Comments and suggestions
Low or no 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) Alkaline lysis was inefficient	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 shifted. This may result in poor lysis conditions because the volumes of Buffers P1, P2, and S3 are not sufficient for setting the plasmid DNA free efficiently. Reduce the culture volume to improve the ratio of biomass to lysis buffer. Also, 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.
c) Buffer P2 or BB precipitated	Redissolve by warming to 37°C.
d) Cell resuspension incomplete	Pelleted cells should be completely resuspended in Buffer P1. Do not add Buffer P2 until an even suspension is obtained.
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	

	Comments and suggestions
Eluate contains residual ethanol et	Ensure that the QIAGEN Plasmid Plus spin column is dried sufficiently (see step 12 on page 20 or step 12 on page 25 or step 13 on page 29 of the protocol).
QIAfilter cartridge clogs during filtration	
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 agitated during incubation	Pour the lysate into the QIAfilter cartridge immediately after addition of Buffer S3 and do not agitate during the 3 minute incubation. Agitation causes the precipitate to be disrupted into tiny particles, instead of forming a layer.
e) 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.
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.
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 minute 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).

	Comments and suggestions
Lysate not clear after filtration	
Precipitate was forced through the QIAfilter cartridge	Filter until all of the lysate has passed through the QIAfilter cartridge, but do not apply extreme force.
QIAGEN Plasmid Plus spin column clogs during binding	
The amount of DNA in the adjusted lysate exceeds the binding capacity of the column	Maximum binding capacity of the column has been reached. Remove residual lysate and perform all subsequent steps in a microcentrifuge.

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 24 QIAGEN Plasmid *Plus* Midi and Maxi and up to 12 QIAGEN Plasmid *Plus* 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 5 and 6 may help to determine an appropriate waste container.

Table 5. Waste volumes

	QIAGEN Plasmid *Plus* Plasmid Mega Kit		QIAGEN Plasmid *Plus* 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 6. 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 to monitor and regulate the vacuum pressure. For more details on the vacuum regulator, please refer to the *QIAvac 24 Plus Handbook*.

A1. 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.

A2. If the QIAvac 24 Plus is used as a stand-alone module, assemble as described in the *QIAvac 24 Plus Handbook* on page 15.

A3. 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)
- **Place the QIAvac 24 Plus on a flat surface. For Mega and Giga preps, place it into the QIAvac Holder.**
- Insert up to 24 QIAGEN Plasmid *Plus* Midi or Maxi spin columns or up to 12 QIAGEN Plasmid *Plus* Mega and Giga spin columns into the luer extensions of the QIAvac 24 Plus (use every second luer slot, below the hole in the QIAvac Holder). Attach a Tube Extender to each column. Close unused positions of the manifold with luer caps and connect the QIAvac 24 Plus to a vacuum source. See Figure 1, page 35.
- **Optional:** VacValves can be used to handle multiple samples with different flow rates. Closing VacValves after liquid has drawn through the first columns ensures that the vacuum pressure remains constant and stable for the remaining samples.
- **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.**
- **Carefully open the main vacuum valve and switch off the vacuum pump.**

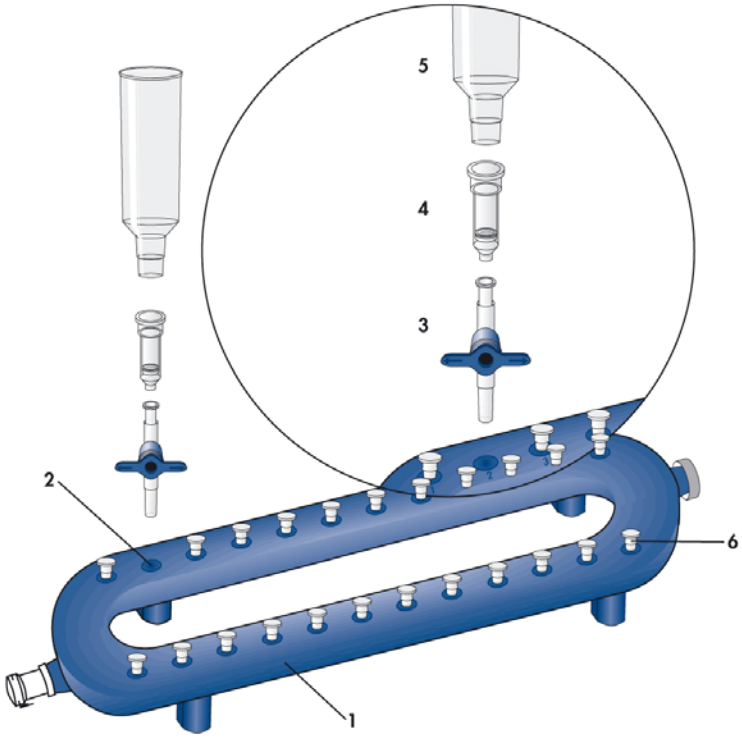


Figure 1. Setting up the QIAvac 24 Plus with QIAGEN Plasmid *Plus* spin columns using VacValves.

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

* Must be purchased separately.

Ordering Information

Product	Contents	Cat. no.
QIAGEN Plasmid *Plus* Midi Kit (25)*	25 QIAGEN Plasmid *Plus* Midi Columns, Extender Tubes, Reagents, Buffers, 25 QIAfilter Midi Cartridges	12943
QIAGEN Plasmid *Plus* Midi Kit (100)*	100 QIAGEN Plasmid *Plus* Midi Columns, Extender Tubes, Reagents, Buffers, 100 QIAfilter Midi Cartridges	12945
QIAGEN Plasmid *Plus* Maxi Kit (25)*	25 QIAGEN Plasmid *Plus* Maxi Columns, Extender Tubes, Reagents, Buffers, 25 QIAfilter Maxi Cartridges	12963
QIAGEN Plasmid *Plus* Maxi Kit (100)*	100 QIAGEN Plasmid *Plus* Maxi Columns, Extender tubes, Reagents, Buffers, 100 QIAfilter Maxi Cartridges	12965
QIAGEN Plasmid *Plus* Mega Kit (5)*	5 QIAGEN Plasmid *Plus* Mega Columns, Extender Tubes, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12981
QIAGEN Plasmid *Plus* Giga Kit (5)*	5 QIAGEN Plasmid *Plus* Giga Columns, Extender Tubes, Reagents, Buffers, 5 QIAfilter Mega-Giga Cartridges	12991
Accessories		
QIAvac 24 Plus	Vacuum manifold for processing 1–24 spin columns: QIAvac 24 Plus Vacuum manifold, Luer Plugs, Quick Couplings	19413
QIAvac Holder	Holder for stabilize QIAGEN Plasmid *Plus* Mega and Giga spin columns and extenders on the QIAvac 24 Plus	19418
VacValves (24)	24 valves for use with the QIAvac 24 and QIAvac 24 Plus	19408

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

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Trademarks: QIAGEN®, EndoFree®, LyseBlue® (QIAGEN Group); DH5® (Life Technologies, Inc.); pBluescript® (Agilent Technologies, Inc.); pGEM® (Promega Corp.).

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Australia ■ techservice-au@qiagen.com

Austria ■ techservice-at@qiagen.com

Belgium ■ techservice-bnl@qiagen.com

Brazil ■ suportetecnico.brasil@qiagen.com

Canada ■ techservice-ca@qiagen.com

China ■ techservice-cn@qiagen.com

Denmark ■ techservice-nordic@qiagen.com

Finland ■ techservice-nordic@qiagen.com

France ■ techservice-fr@qiagen.com

Germany ■ techservice-de@qiagen.com

Hong Kong ■ techservice-hk@qiagen.com

India ■ techservice-india@qiagen.com

Ireland ■ techservice-uk@qiagen.com

Italy ■ techservice-it@qiagen.com

Japan ■ techservice-jp@qiagen.com

Korea (South) ■ techservice-kr@qiagen.com

Luxembourg ■ techservice-bnl@qiagen.com

Mexico ■ techservice-mx@qiagen.com

The Netherlands ■ techservice-bnl@qiagen.com

Norway ■ techservice-nordic@qiagen.com

Singapore ■ techservice-sg@qiagen.com

Sweden ■ techservice-nordic@qiagen.com

Switzerland ■ techservice-ch@qiagen.com

UK ■ techservice-uk@qiagen.com

USA ■ techservice-us@qiagen.com

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