June 2015

CompactPrep® Plasmid Purification Handbook

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

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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Contents

Kit Contents

CompactPrep Plasmid Kit	Midi (25)	Maxi (25)
Catalog no.	**12843**	**12863**
CompactPrep Columns	25 Midi Columns	25 Maxi Columns
Tube Extenders (10 ml)	25	–
Tube Extenders (20 ml)	–	25
Collection Tubes (2 ml)	25	25
Buffer P1	2 x 50 ml	3 x 50 ml
Buffer P2	4 x 20 ml	150 ml
Buffer S3	70 ml	2 x 70 ml
Buffer BB	70 ml	2 x 70 ml
Buffer PE (concentrate)	6 ml	6 ml
Buffer EB	15 ml	15 ml
RNase A*	2 x 5 mg	3 x 5 mg
LyseBlue®	2 x 50 µl	3 x 50 µl
Quick-Start Protocol	1	1

* Provided as a 10 mg/ml solution.

Storage

CompactPrep Plasmid Kits should be stored dry at room temperature (15–25°C). Kits can be stored for up to 2 years 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

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

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

Safety Information

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

Quality Control

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

Introduction

QIAGEN CompactPrep Plasmid Kits provide a novel method for very fast (<20 minutes) large-scale plasmid preparation, without the need for large volume centrifuges. The procedure is based on a novel, non-chaotropic binding chemistry, reducing the total preparation volume to miniprep scale. All protocol steps after cell harvest can be performed at the bench using QIAfilter cartridges, a vacuum manifold, and a standard microcentrifuge. CompactPrep Plasmid Kits do not contain QIAfilter cartridges and require lysate clearing by centrifugation. The unique kit chemistry and design of the CompactPrep column ensure large-scale plasmid prep using a microspin column format.

CompactPrep Plasmid Kits provide molecular biology grade DNA, highly suited for routine applications such as sequencing, enzymatic modification, cloning, and transfection into robust cell lines such as HeLa, COS-7, CHO, HEK 293, and NIH 3T3. A higher DNA quality may be required for transfection into primary or sensitive cell lines.

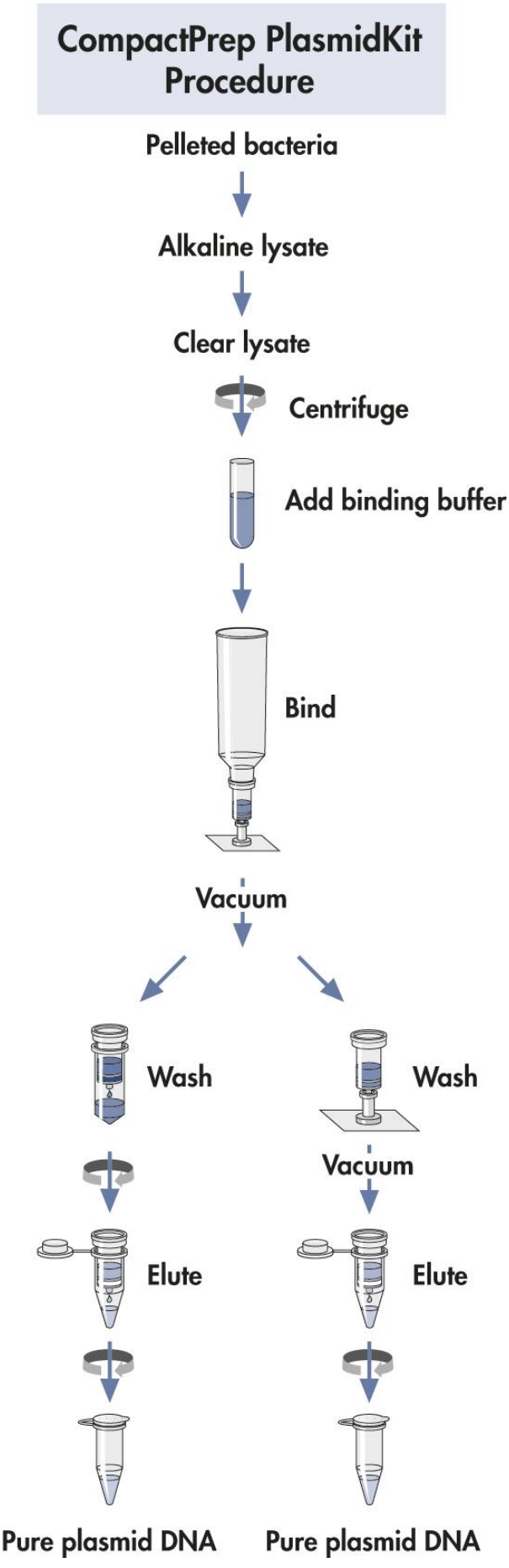
QIAGEN offers the most comprehensive portfolio of tailored plasmid purification kits for any scale, throughput, or downstream application. Select the optimum kit for your requirements by visiting our online selection guide at www.qiagen.com/products/plasmid/selectionguide.

Principle and procedure

The CompactPrep Plasmid Kit protocol is based on a modified alkaline lysis procedure. A novel binding buffer (Buffer BB) is added to the cleared lysate to optimize plasmid DNA binding under non-chaotropic conditions to the membrane of the CompactPrep column. The combination of CompactPrep columns with the newly developed binding chemistry uniquely allows large-scale yields of plasmid DNA to be obtained using a microspin column format. A vacuum manifold (e.g., QIAvac 24 Plus, cat. no. 19413) is used to draw the cleared lysate and subsequent wash buffer through the CompactPrep column. DNA is eluted in low volumes (100 µl Midi; 200 µl Maxi) of elution buffer (Buffer EB) by centrifugation using a microcentrifuge. The highly concentrated DNA (typically >1 µg/µl) is ready for immediate 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 16.


CompactPrep PlasmidKit Procedure
Pelleted bacteria
Alkaline lysate
Clear lysate
Centrifuge
Add binding buffer
Bind
Vacuum
Wash
Wash
Vacuum
Elute
Elute
Pure plasmid DNA
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 safety data sheets (SDSs), 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
- Microcentrifuge
- Vacuum manifold (e.g., QIAvac 24 Plus, cat. no. 19413)
- **Optional**: Water or TE buffer for elution
- Refrigerated centrifuge capable of ≥20,000 x *g* with rotor for the appropriate centrifuge tubes or bottles

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.

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 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 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 that is too high, to 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.

Analytical gel analysis

The success of the plasmid purification procedure can be monitored on an analytical gel (see Figure 2, page 31). 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 (see page 31).

Convenient stopping points in protocols

For all protocols, the purification procedure can be stopped and continued later by freezing the cell pellets obtained by centrifugation. The frozen cell pellets can be stored at –20°C for several weeks. These stopping points are indicated by the symbol ⊗.

Using LyseBlue reagent

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

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

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

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

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

Upon addition of neutralization buffer (Buffer 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.

Centrifugation

- All microcentrifugation steps are carried out at 10,000 x *g* (approximately 13,000 rpm) in a conventional tabletop microcentrifuge.
- CompactPrep Plasmid Kits require a refrigerated centrifuge capable of ≥20,000 x *g* and a rotor for the appropriate centrifuge tubes or bottles for lysate clearing.

Vacuum manifolds

Use of a vacuum manifold is required to draw the DNA solution into the CompactPrep column.

A waste disposal vessel allowing sufficient volume for the amount of preps run should be attached to the vacuum manifold.

QIAvac 24 Plus vacuum manifold

- Remove the collection tube from the CompactPrep column. Do not discard! Insert up to 24 CompactPrep Midi or Maxi columns into the luer extensions of the QIAvac 24 Plus. Attach a tube extender (10 ml) to each CompactPrep Midi column and a tube extender (20 ml) to each CompactPrep Maxi column. Close unused positions of the manifold with luer caps and connect the QIAvac 24 Plus to a vacuum source. See Figure 1, page 18.
- **Optional**: VacValves can be used to handle multiple samples with different flow rates. Closing VacValves after liquid has been drawn through the first columns ensures that the vacuum pressure remains constant and stable for the remaining samples.

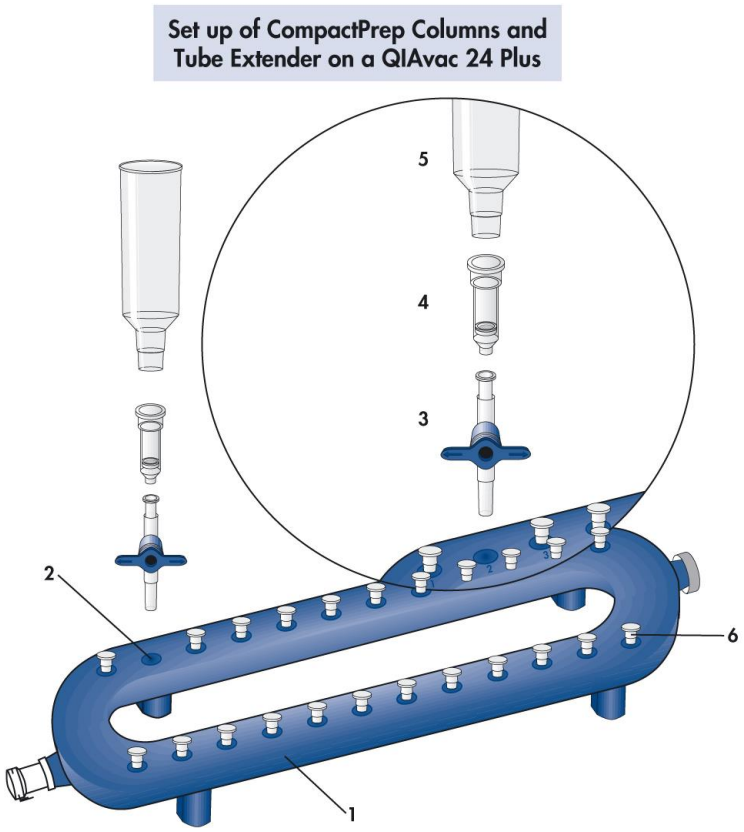


Figure 1. Setting up the QIAvac 24 Plus with CompactPrep columns using VacValves

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

* Must be purchased separately.

Other vacuum manifolds

Follow the supplier's instructions. Insert each CompactPrep column into a luer connector.

Protocol: Purification of Plasmid DNA using CompactPrep Plasmid Kits

This protocol is designed for the preparation of up to ■ 200 µg of high-copy plasmid DNA using the CompactPrep Plasmid Midi Kit, or up to ▲ 750 µg using the CompactPrep Plasmid Maxi Kit. In this protocol centrifugation is used to clear bacterial lysates.

Table 4. Maximum recommended culture volumes

	CompactPrep Plasmid Midi	CompactPrep Plasmid Maxi
High-copy plasmid*	25	100
Low-copy plasmid*†	50	250

* For high-copy plasmids, expected yields are ■ 100–200 µg for the CompactPrep Plasmid Midi Kit and ▲ 300–750 µg for the CompactPrep Plasmid Maxi Kit. For low-copy plasmids, expected yields are ■ 30–100 µg for the CompactPrep Plasmid Midi Kit and ▲ 50–250 µg for the CompactPrep Plasmid Maxi Kit using these culture volumes.

† Low-copy plasmids can be efficiently purified using CompactPrep Plasmid Kits, however, growing bacteria to 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.

Important points before starting

- Text marked with a ■ denotes values for the CompactPrep Plasmid Midi Kit; text marked with a ▲ denotes values for the CompactPrep Plasmid Maxi Kit.
- **Optional**: remove samples at the indicated steps to monitor the procedure on an analytical gel (see appendix, page 31).

Things to do before starting

- Add the provided RNase A solution to Buffer P1 before use. Use 1 vial RNase A (centrifuge briefly before use) per bottle Buffer P1 for a final concentration of 100 µg/ml.
- 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.

■ **Optional**: Add the provided LyseBlue reagent to Buffer P1 and mix before use. Use 1 vial LyseBlue reagent per bottle Buffer P1 for a final dilution of 1:1000 (e.g., 10 µl LyseBlue into 10 ml Buffer P1). LyseBlue provides visual identification of optimum buffer mixing, thereby preventing the common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA, and cell debris. For more details see "Using LyseBlue reagent" on page 16.

Procedure

1. **Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 ml LB medium containing the appropriate selective antibiotic. Incubate for approximately 8 h at 37°C with vigorous shaking (approximately 300 rpm).**

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

2. **Dilute the starter culture 1/500 to 1/1000 into selective LB medium. For high-copy plasmids inoculate ■ 25 ml or ▲ 100 ml medium. For low-copy plasmids, inoculate ■ 50 ml or ▲ 250 ml medium. Grow at 37°C for 12–16 hours with vigorous shaking (approximately 300 rpm). Do not increase the culture volume, as this will lead to a decrease in DNA yield and quality.**

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

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

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

4. **Resuspend the bacterial pellet in ■ 2 ml or ▲ 5 ml Buffer P1.**

   For efficient lysis it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. Ensure that RNase A has been added to Buffer P1. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

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

5. **Add ■ 2 ml or ▲ 5 ml Buffer P2, mix thoroughly by vigorously inverting the sealed tube 4–6 times, and incubate at room temperature (15–25°C) for 3 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.

6. **Add ■ 2 ml or ▲ 5 ml Buffer S3 to the lysate, and mix immediately by vigorously inverting 4–6 times. Proceed directly to step 7. Do not incubate the lysate on ice.**

   After addition of Buffer S3, a fluffy white precipitate containing genomic DNA, proteins, cell debris, and KDS* becomes visible. 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.

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

   Before loading the centrifuge, the sample should be mixed again. Centrifugation should be performed in non-glass tubes (e.g., polypropylene). After centrifugation the supernatant should be clear.

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

   This second centrifugation step should be carried out to avoid applying suspended or particulate material to the CompactPrep column. Suspended material (causing the sample to appear turbid) can clog the CompactPrep column.

   Optional: Remove a ■ 35 µl or ▲ 60 µl sample of the cleared lysate and save for an analytical gel to determine whether growth and lysis conditions were optimal.

9. **During incubation, prepare the vacuum manifold and CompactPrep Midi or Maxi columns (see vacuum manifolds, pages 17–18).**

* Potassium dodecyl sulfate.

10. **Add ■ 2 ml or ▲ 5 ml Buffer BB, to the lysate. Mix by inverting 4–6 times and transfer the adjusted lysate into a tube extender attached to the CompactPrep column.**

    Buffer BB may precipitate when added to the cleared lysate after centrifugation at 4°C. Allow the lysate–Buffer BB solution to warm up and become clear before proceeding with the next step. Alternatively, allow cleared lysate to warm to room temperature before adding Buffer BB.

11. **Switch on vacuum source to draw the solution through the CompactPrep column, and then switch off vacuum source.**

12. **To wash the DNA using a microcentrifuge, proceed with step 12a. Alternatively, to wash the DNA using a vacuum manifold proceed to step 12b.**

12a. **To wash the DNA using a microcentrifuge:**

    Discard the tube extenders and place the CompactPrep column into a 2 ml collection tube provided. Wash the CompactPrep column by adding 0.7 ml Buffer PE and centrifuging for 30–60 s. Discard the flow-through, and centrifuge for an additional 1 min to remove residual wash buffer.

12b. **To wash the DNA using a vacuum manifold:**

    Discard the tube extenders. Add 0.7 ml Buffer PE to the column and switch on the vacuum manifold. To completely remove residual wash buffer, continue to apply vacuum for a further 10 min after the solution has been drawn through.

    Note: The residual wash buffer can be removed by centrifugation of the CompactPrep column for 1 min in a microcentrifuge.

13. **Place the CompactPrep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add ■ 100 µl or ▲ 200 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the CompactPrep column, let stand for 1 min, then centrifuge for 1 min.**

    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. To ensure accuracy, make sure the absorbance readings fall into the linear range of your method (e.g., between 0.1 and 1.0 for spectrophotometric OD).

Agarose gel analysis

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 procedure where the problem occurred (see page 31).

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 P3 to achieve homogeneous suspensions. Use LyseBlue to visualize efficiency of mixing.
c) Insufficient lysis for low-copy plasmids	For low-copy plasmid preparations, doubling the volumes of Buffers P1, P2, S3, and BB may help to increase plasmid yield and quality.
d) Buffer P2 or Buffer BB precipitated	Redissolve by warming to 37°C.
e) Cell Resuspension incomplete	Pelleted cells should be completely resuspended in Buffer P1. Do not add Buffer P2 until an even suspension is obtained.

	Comments and suggestions
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 (see step 13 [page 22], or step 12 [page 27] of the protocol).
CompactPrep 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: Agarose Gel Analysis of the Purification Procedure

DNA yields and quality can be readily analyzed by agarose gel electrophoresis. Poor yields and quality can be caused by a number of different factors. To determine the stage of the procedure where the problem occurred, save 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.

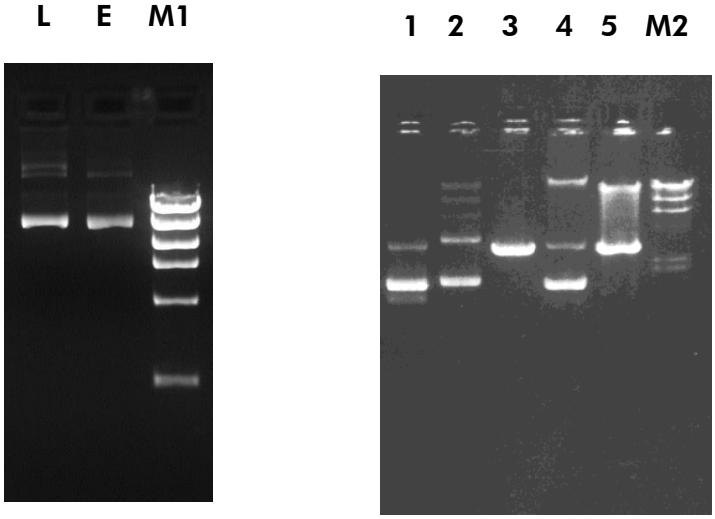


Figure 2. Agarose gel analysis of the plasmid purification procedure.

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 29–30. 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 safety data sheets (SDSs), available from the product supplier.

L: Cleared lysate containing supercoiled and open circular plasmid DNA and degraded RNA.

E: The eluate containing pure plasmid DNA with no other contaminating nucleic acids.

M1: kb ladder (1, 2, 3, 4, 6, 10 kb).

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 Buffer P3). Genomic DNA contamination can easily be identified by digestion of the sample with *Eco*RI. A smear is observed, in contrast to the linear band seen after digestion of multimeric plasmid forms.

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

M2: Lambda DNA digested with *Hin*dIII.

Ordering Information

Product	Contents	Cat. no.
CompactPrep Plasmid Midi Kit (25)*	25 CompactPrep Midi Columns, Extender tubes, Reagents, Buffers	12843
CompactPrep Plasmid Maxi Kit (25)*	25 CompactPrep Maxi Columns, Extender tubes, Reagents, Buffers	12863
Accessories		
QIAvac 24 Plus	Vacuum manifold for processing 1–24 spin columns: QIAvac 24 Plus Vacuum manifold, Luer Plugs, Quick Couplings	19413

* CompactPrep Plasmid 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

Notes

Limited License Agreement for CompactPrep Plasmid Kits

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.
4. QIAGEN specifically disclaims any other licenses, expressed or implied other than those expressly stated.
5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. QIAGEN may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

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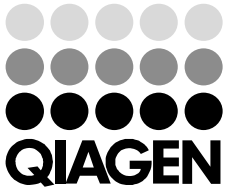

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