May 2004

QIAprep® Miniprep Handbook

For purification of

Plasmid DNA
Large plasmids (>10 kb)
Low-copy plasmids and cosmids
Plasmid DNA prepared by other methods

QIAGEN

Contents

Kit Contents

QIAprep® Spin Miniprep Kits	(50)	(250)
Catalog no.	**27104**	**27106**
QIAprep Spin Columns	50	250
Buffer P1	20 ml	70 ml
Buffer P2	20 ml	70 ml
Buffer N3*	30 ml	140 ml
Buffer PB*	30 ml	150 ml
Buffer PE (concentrate)	2 x 6 ml	55 ml
Buffer EB	15 ml	55 ml
RNase A†	20 µl	70 µl
Collection Tubes (2 ml)	50	250
Handbook	1	1

QIAprep 8 Miniprep Kits	(50)
Catalog no.	**27144**
QIAprep 8 Strips	50
Buffer P1	140 ml
Buffer P2	140 ml
Buffer N3*	250 ml
Buffer PB*	500 ml
Buffer PE (concentrate)	2 x 100 ml
Buffer EB	2 x 55 ml
RNase A†	140 µl
Collection Microtubes (1.2 ml)	55 x 8
Caps for QIAprep Strips	55 x 8
Caps for Collection Microtubes	55 x 8
Handbook	1

* Buffers N3 and PB contain chaotropic salts which are irritants and not compatible with disinfecting agents containing bleach. Take appropriate laboratory safety measures and wear gloves when handling. See page 9 for further information.

† Provided as a 100 mg/ml solution.

QIAprep 8 Turbo Miniprep Kit	(10)	(50)
Catalog no.	**27152**	**27154**
TurboFilter® 8 Strips	10	50
QIAprep 8 Strips	10	50
Buffer P1	40 ml	125 ml
Buffer P2	40 ml	125 ml
Buffer N3*	60 ml	2 x 125 ml
Buffer PB*	100 ml	500 ml
Buffer PE (concentrate)	2 x 20 ml	2 x 100 ml
Buffer EB	55 ml	2 x 55 ml
RNase A†	40 µl	125 µl
Collection Microtubes (1.2 ml)	13 x 8	55 x 8
Caps for QIAprep Strips	13 x 8	55 x 8
Caps for Collection Microtubes	13 x 8	55 x 8
Handbook	1	1

* Buffers N3 and PB contain chaotropic salts which are irritants and not compatible with disinfecting agents containing bleach. Take appropriate laboratory safety measures and wear gloves when handling. See page 9 for further information.

† Provided as a 100 mg/ml solution.

QIAprep 96 Turbo Miniprep Kit	(4)	(24)
Catalog no.	**27191**	**27193**
TurboFilter 96 Plates	4	24
QIAprep 96 Plates	4	24
Buffer P1	125 ml	1 x 700 ml, 1 x 125 ml
Buffer P2	125 ml	1 x 700 ml, 1 x 125 ml
Buffer N3*	2 x 80 ml	1 x 1000 ml, 1 x 80 ml
Buffer PB*	500 ml	6 x 500 ml
Buffer PE (concentrate)	2 x 100 ml	5 x 200 ml, 2 x 100 ml
Buffer EB	2 x 55 ml	1 x 55 ml, 2 x 250 ml
RNase A†	125 µl	1 x 125 µl, 1 x 700 µl
Tape Pads	1	6
Rack of Collection Microtubes (1.2 ml)	4	24
Caps for Collection Microtubes	55 x 8	6 x 55 x 8
Flat-Bottom Blocks and Lids	4	24
Handbook	1	1

* Buffers N3 and PB contain chaotropic salts which are irritants and not compatible with disinfecting agents containing bleach. Take appropriate laboratory safety measures and wear gloves when handling. See page 9 for further information.

† Provided as a 100 mg/ml solution.

Storage

QIAprep Miniprep Kits should be stored dry at room temperature (15–25°C). Kits can be stored for up to 12 months without showing any reduction in performance and quality. For longer storage these kits can be kept at 2–8°C. If any precipitate forms in the buffers after storage at 2–8°C it should be redissolved by warming the buffers to 37°C before use.

After addition of RNase A, Buffer P1 is stable for six months when stored at 2–8°C. RNase A stock solution can be stored for two years at room temperature.

Quality Control

As part of the stringent QIAGEN quality assurance program, the performance of QIAprep Miniprep Kits is monitored routinely on a lot-to-lot basis. DNA binding capacity is tested by determining the recovery obtained with 10 µg and 20 µg of various plasmid DNAs. The DNA yield of QIAprep Miniprep Kits is tested by preparing high-copy plasmid DNA from 1–5 ml overnight culture in LB medium and assaying the DNA yield spectrophotometrically. The quality of isolated DNA is checked by restriction digestion, agarose gel electrophoresis, and spectrophotometry.

Product Use Limitations

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

Product Warranty and Satisfaction Guarantee

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

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

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 molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding QIAprep Miniprep 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 call one of the QIAGEN Technical Service Departments or local distributors (see back cover).

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.

CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Buffers N3 and PB contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach.

If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

The following risk and safety phrases apply to QIAprep Miniprep Kits.

Buffer N3

Contains guanidine hydrochloride, acetic acid: harmful, irritant. Risk and safety phrases:* R22-36/38, S13-23-26-36/37/39-46.

Buffer P2

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

Buffer PB

Contains guanidine hydrochloride, isopropanol: harmful, flammable, irritant. Risk and safety phrases:* R10-22-36/38, S13-23-26-36/37/39-46

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

* R10: Flammable; R22: Harmful if swallowed; R36/38: Irritating to eyes and skin; R42/43: May cause sensitization by inhalation and skin contact; 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 protecting clothing and gloves; S36/37/39: Wear suitable protective clothing, gloves and eye/face protection; S46: If swallowed seek medical advice immediately and show this container or label.

Introduction

The QIAprep Miniprep system provides a fast, simple, and cost-effective plasmid miniprep method for routine molecular biology laboratory applications. QIAprep Miniprep Kits use silica-gel–membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. Plasmid DNA purified with QIAprep Miniprep Kits is immediately ready for use. Phenol extraction and ethanol precipitation are not required, and high-quality plasmid DNA is eluted in a small volume of Tris buffer (included in each kit) or water. The QIAprep system consists of four products with different handling options to suit every throughput need (Table 1):

Low throughput

The **QIAprep Spin Miniprep Kit** is designed for quick and convenient processing of 1–24 samples simultaneously in less than 30 minutes. QIAprep spin columns can be used in a microcentrifuge or on any vacuum manifold with luer connectors e.g., QIAvac 24, or QIAvac 6S with QIAvac Luer Adapters.

Medium throughput

The **QIAprep 8 Miniprep Kit** uses 8-well strips on QIAvac 6S. This kit is suitable for medium-throughput needs, and allows up to 48 minipreps to be performed simultaneously in approximately 40 minutes. The **QIAprep 8 Turbo Miniprep Kit** streamlines plasmid purification even further, using TurboFilter 8 strips. These allow rapid clearing of bacterial lysates with the use of filtration rather than centrifugation. Up to 48 minipreps can be completed in just 30 minutes. The **QIAprep 8 Turbo BioRobot® Kit** is a special kit format optimized for use with BioRobot systems. Up to 48 minipreps can be completed in 50 minutes with walkaway automation.

High throughput

The **QIAprep 96 Turbo Miniprep Kit** offers the QIAprep Turbo advantage in 96-well format, allowing up to 96 minipreps to be performed simultaneously in less than 45 minutes on the QIAvac 96. The QIAprep Turbo Miniprep Kit is the perfect choice for high-quality, high-throughput DNA minipreps. The **QIAprep 96 Turbo BioRobot Kit** offers automated high-throughput preparation of high-purity plasmid DNA on BioRobot systems. Up to 96 minipreps can be processed in 70 minutes.

Applications using QIAprep purified DNA

Plasmid DNA prepared using the QIAprep system is suitable for a variety of routine applications including:

- Restriction enzyme digestion
- Radioactive and fluorescent sequencing
- Library screening
- Sequencing
- Ligation and transformation

Table 1. Comparison of QIAprep Products

Product	QIAprep Spin Miniprep Kit	QIAprep 8 Miniprep Kit	QIAprep 8 Turbo Miniprep Kit	QIAprep 96 Turbo Miniprep Kit
Format	Spin columns	8-well strips	8-well strips	96-well plates
Equipment	Microcentrifuge OR QIAvac 24, OR QIAvac 6S with QIAvac Luer Adapter Set*	QIAvac 6S	QIAvac 6S†	QIAvac 96†
Throughput	1–24	8–48	8–48	48–96
Clearing of lysates	Microcentri-fugation	Microcentri-fugation	Rapid vacuum filtration	Rapid vacuum filtration
Time	12 minipreps in 26 min (centrifuge) or 20 min (vacuum)	24 minipreps in 25 min	24 minipreps in 20 min	48 minipreps in 35 min
	24 minipreps in 30 min (centrifuge or vacuum)	48 minipreps in 34 min	48 minipreps in 30 min	96 minipreps in 45 min
Capacity of column reservoir	800 µl	1 ml	1 ml	1 ml
DNA binding capacity of membrane	20 µg dsDNA	20 µg dsDNA	20 µg dsDNA	20 µg dsDNA
Recovery	85–95%‡	85–95%‡	85–95%‡	85–95%‡
Minimum elution buffer volume	50 µl	75 µl	75 µl	75 µl
Culture volume for high-copy plasmids	1–5 ml	1–5 ml	1–5 ml	1–5 ml
Culture volume for low-copy plasmids	1–10 ml	1–10 ml	1–10 ml	1–10 ml

* Or any other vacuum manifold with luer connectors. † BioRobot Kit formats available for QIAprep 8 Turbo and QIAprep 96 Turbo. ‡ Depends on elution volume (see "DNA yield", page 14).

Principle

The QIAprep miniprep procedure is based on alkaline lysis of bacterial cells followed by adsorption of DNA onto silica in the presence of high salt (1). The unique silica-gel membrane used in QIAprep Miniprep Kits completely replaces glass or silica slurries for plasmid minipreps.

The procedure consists of three basic steps:

- preparation and clearing of a bacterial lysate
- adsorption of DNA onto the QIAprep membrane
- washing and elution of plasmid DNA

All steps are performed without the use of phenol, chloroform, CsCl, ethidium bromide, and without alcohol precipitation.

Alkaline lysis of bacteria

The QIAprep miniprep procedure uses the modified alkaline lysis method of Birnboim and Doly (2). Bacteria are lysed under alkaline conditions, and the lysate is subsequently neutralized and adjusted to high-salt binding conditions in one step, ready for purification on the QIAprep silica-gel membrane. For more details on growth of bacterial cultures and alkaline lysis, please refer to Appendix A on pages 41–44.

Lysate clearing

TurboFilter modules provided in QIAprep 8 and 96 Turbo Miniprep Kits eliminate the need for lysate centrifugation. Following alkaline lysis of bacterial cultures, the crude lysates are loaded directly onto the TurboFilter 8 strip or TurboFilter 96 plate. Denatured and precipitated cellular components are removed by filtration through the TurboFilter membrane. Particle-free filtrates flow directly into the wells of the QIAprep module.

In the QIAprep Spin and QIAprep 8 miniprep procedures, lysates are cleared by centrifugation.

DNA adsorption to the QIAprep membrane

QIAprep columns, strips, and plates use a silica-gel membrane for selective adsorption of plasmid DNA in high-salt buffer and elution in low-salt buffer. The optimized buffers in the lysis procedure combined with the unique silica-gel membrane ensure that only DNA will be adsorbed, while RNA, cellular proteins, and metabolites are not retained on the membrane but are found in the flow-through.

Washing and elution of plasmid DNA

Endonucleases are efficiently removed by a brief wash step with Buffer PB. This step is essential when working with *endA*$^+$ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, to ensure that plasmid DNA is not degraded. The Buffer PB wash step is also necessary when purifying low-copy plasmids, where large culture volumes are used.

Salts are efficiently removed by a brief wash step with Buffer PE. High-quality plasmid DNA is then eluted from the QIAprep column with 50–100 µl of Buffer EB or water. The purified DNA is ready for immediate use in a range of applications — no need to precipitate, concentrate, or desalt.

Note: Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH value is within this range. Store DNA at –20°C when eluted with water since DNA may degrade in the absence of a buffering agent.

DNA yield

Plasmid yield with the QIAprep miniprep system varies depending on plasmid copy number per cell (see page 41), the individual insert in a plasmid, factors that affect growth of the bacterial culture (see pages 41–44), the elution volume (Figure 1), and the elution incubation time (Figure 2, page 15). A 1.5 ml overnight culture can yield from 5 to 15 µg of plasmid DNA (Table 2, page 15). To obtain the optimum combination of DNA quality and concentration, we recommend using Luria-Bertani (LB) medium for growth of cultures (for composition see page 43), eluting plasmid DNA in a volume of 50 µl, and performing a short incubation after addition of the elution buffer.

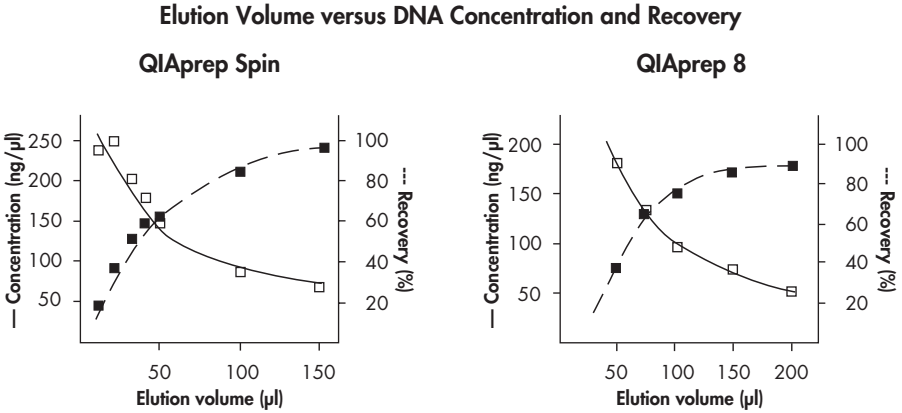


Figure 1 10 µg pUC18 DNA was purified using QIAprep Spin and QIAprep 8 Miniprep protocols, and eluted with the indicated volumes of Buffer EB.

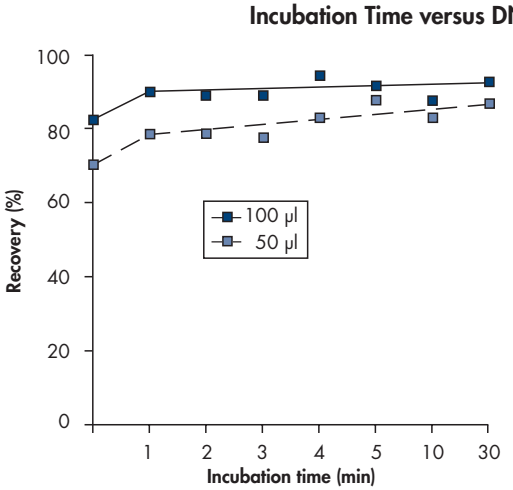


Figure 2 10 µg pBluescript® DNA was purified using the QIAprep Spin Miniprep protocol and eluted after the indicated incubation times with Buffer EB.

Table 2. Effect of Incubation During Elution on Plasmid Yield and Concentration

Culture media	Elution volume	Without incubation Yield	Without incubation Rel. conc.	With incubation Yield	With incubation Rel. conc.
LB (containing 10 g/liter NaCl)	50 µl	9.9 µg	1.0	11.5 µg	1.2
	100 µl	11.5 µg	0.6	14.4 µg	0.8
LB (containing 5 g/liter NaCl)	50 µl	8.5 µg	1.0	9.5 µg	1.1
	100 µl	10.2 µg	0.6	11.8 µg	0.7

The effect on DNA yield and relative concentration (rel. conc.) of different elution volumes, different culture media, and a 5 minute incubation prior to centrifugation. QIAprep Spin Miniprep Kit was used to purify DNA from 1.5 ml LB overnight cultures of XL1-Blue containing pBluescript. An elution volume of 50 µl without incubation was used as standard. A 50 µl elution volume with a short incubation after addition of the elution buffer resulted in the optimal combination of DNA yield and concentration.

QIAvac Vacuum Manifolds

QIAvac 24 Manifold

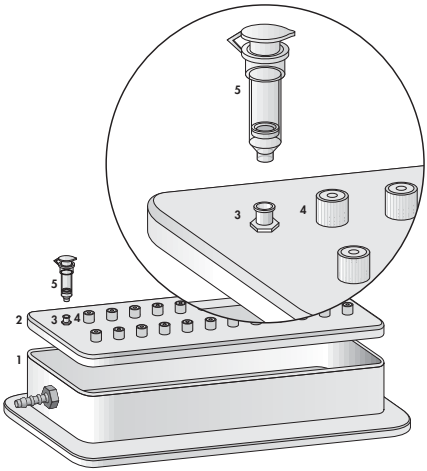


Figure 3 Components of the QIAvac 24 manifold.

1. QIAvac 24 base
2. QIAvac 24 lid
3. Luer extension of QIAvac 24
4. Luer extension closed with luer cap
5. QIAprep spin column*

* Not included with QIAvac 24. Included in appropriate kits.

QIAvac 6S Manifold

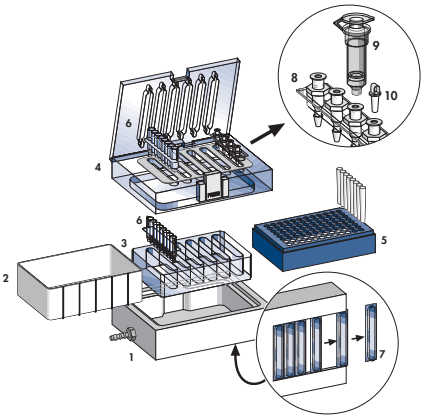


Figure 4 Components of the QIAvac 6S manifold.

1. QIAvac base, which holds a waste tray, a strip holder, or a microtube rack
2. Waste tray
3. QIAvac strip holder to hold 8-well strips
4. QIAvac top plate with slots for 8-well strips or QIAvac Luer Adapters
5. Microtube rack
6. 8-well strip*
7. Blanks to seal unused slots
8. QIAvac Luer Adapter†
9. QIAprep spin column*
10. Plug to seal unused luer connectors†

QIAvac 96 Manifold

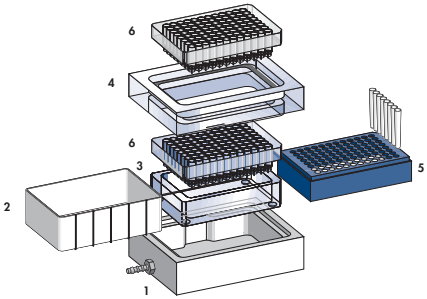


Figure 5 Components of the QIAvac 96 manifold.

1. QIAvac base, which holds a waste tray, a plate holder, or a microtube rack
2. Waste tray
3. Plate holder (shown with 96-well plate)
4. QIAvac 96 top plate with aperture for 96-well plate
5. Microtube rack
6. 96-well plate‡

* Not included with QIAvac 6S. Included in appropriate kits. † Not included with QIAvac 6S. Must be purchased separately. ‡ Not included with QIAvac 96. Included in QIAprep 96 Turbo Miniprep Kits.

Guidelines for QIAvac Manifolds

QIAvac 24, QIAvac 6S, and QIAvac 96 facilitate DNA minipreps by providing a convenient modular vacuum manifold for use with the QIAprep system. The following recommendations should be followed when handling QIAvac manifolds.

- QIAvac manifolds operate with a house vacuum, vacuum pump, or water aspirator.
- Always store QIAvac manifolds clean and dry. To clean, simply rinse all components with water and dry with paper towels. Do not air dry, as the screws may rust and need to be replaced. Do not use abrasives or solvents.
- Always place the QIAvac manifold on a secure bench top or work area. If dropped, the manifold may crack.
- The components of QIAvac manifolds are not resistant to ethanol, methanol, or other organic solvents (Table 3). Do not bring solvents into contact with the vacuum manifold. If solvents are spilled on the unit, rinse thoroughly with distilled water. Ensure that no residual Buffer PE remains in the vacuum manifold.
- To ensure consistent performance, do not apply silicone or vacuum grease to any part of a QIAvac manifold. The spring lock on the top plate and the self-sealing gasket (QIAvac 6S and QIAvac 96) provide an airtight seal when vacuum is applied to the assembled unit. To maximize gasket lifetime, rinse the gasket free of salts and buffers after each use and dry with paper towels before storage.

Table 3. Chemical-Resistance Properties of QIAvac Manifolds

Resistant to:	Not resistant to:	
Chlorine bleach (12%)	Acetic acid	Benzene
Hydrochloric acid	Acetone	Chloroform
Sodium chloride	Chromic acid	Ethers
Sodium hydroxide	Phenol	Toluene
Urea	Concentrated alcohols	

Important Notes

Please read the following notes before starting any of the QIAprep procedures.

Buffer notes:

- Add the provided RNase A solution to Buffer P1, mix, and store at 2–8°C.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- Check Buffers P2 and N3 before use for salt precipitation. Redissolve any precipitate by warming to 37°C. Do not shake Buffer P2 vigorously.
- Close the bottle containing Buffer P2 immediately after use to avoid acidification of Buffer P2 from CO_2 in the air.
- Buffers P2, N3, and PB contain irritants. Wear gloves when handling these buffers.

Centrifugation notes:

- All centrifugation steps are carried out at 13,000 rpm (~17,900 x *g*) in a conventional, table-top microcentrifuge.

Vacuum notes:

- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during manipulations.
- Wear safety glasses when working near a manifold under pressure.
- For safety reasons, do not use 96-well plates that have been damaged in any way.
- For QIAprep 8, QIAprep 8 Turbo, and QIAprep 96 Turbo miniprep procedures, the negative pressure (vacuum) should be regulated before beginning the procedure by applying the vacuum to the appropriate number of **empty** QIAprep modules (indicated in Table 4) on the QIAvac manifold.

  The vacuum pressure is the pressure differential between the inside of the manifold and the atmosphere (standard atmospheric pressure: 1013 millibar or 760 mm Hg) and can be measured using a vacuum regulator (see ordering information, page 47). Vacuum recommendations are given in negative units (Table 4) to indicate the required reduction in pressure with respect to the atmosphere. Table 5 provides pressure conversions to other units.
- Use of a vacuum pressure lower than recommended may reduce DNA yield and purity.

Table 4. Regulation of Vacuum Pressures for QIAprep Multiwell Procedures

Procedure	Vacuum manifold	Module used for checking pressure*	Vacuum pressure‡ mbar	mm Hg
QIAprep 8	QIAvac 6S	QIAprep 8 strip(s)†	–100 to –530	–75 to –400
QIAprep 8 Turbo	QIAvac 6S	QIAprep 8 strip(s)†	–100 to –530	–75 to –400
QIAprep 96 Turbo	QIAvac 96	QIAprep 96 plate	–40 to –200	–30 to –150

* Pressure should be regulated using empty modules on the manifold.

† Regulate the vacuum using the number of 8-well strips that will be used in the purification.

‡ Values apply to empty modules on QIAvac. During the working procedure the vacuum may exceed the values indicated.

Table 5. Pressure Conversions

To convert from millibars (mbar) to	Multiply by:
Millimeters of mercury (mm Hg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atm)	0.000987
Pounds per square inch (psi)	0.0145

Elution notes:

- Ensure that the elution buffer is dispensed directly onto the center of the QIAprep membrane for optimal elution of DNA. Average eluate volume is 45 µl from an elution-buffer volume of 50 µl (QIAprep spin procedures), and 60 µl from an elution-buffer volume of 100 µl (QIAprep multiwell procedures).
- For increased DNA yield, use a higher elution-buffer volume. For increased DNA concentration, use a lower elution-buffer volume (see "DNA yield", pages 14–15).
- If water is used for elution, make sure that its pH is between 7.0 and 8.5. Elution efficiency is dependent on pH and the maximum elution efficiency is achieved within this range. A pH <7.0 can decrease yield.

  Note: Store DNA at –20°C when eluted with water, as DNA may degrade in the absence of a buffering agent.
- DNA can also be eluted in TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

Multichannel pipet recommendations

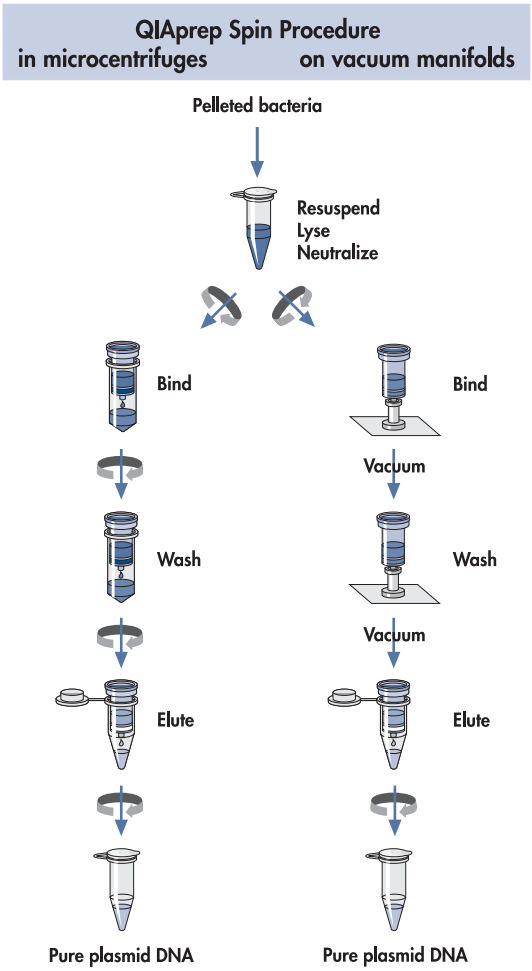
Many steps of the QIAprep 8 procedure and the QIAprep 8 and 96 Turbo procedures require repeated pipetting, and a reservoir or multichannel pipet can greatly facilitate liquid handling. The Matrix Impact® cordless multichannel pipet can be purchased with an optional expandable tip-spacing system for direct liquid transfer from tubes to microtiter plates.

These can be purchased from Matrix Technologies Corporation: www.matrixtechcorp.com .

Pipet tip recommendations

Some standard 1 ml pipet tips are not easily accommodated in the flat-bottom blocks that are used in the QIAprep 96 Turbo Miniprep protocol. When pipetting into flat-bottom blocks, we recommend using pipet tips with 1.25 ml or 1.5 ml fill volume, such as:

- Matrix pipet tips (cat. no. 8051) for use with the Matrix pipet mentioned above. These can be purchased from the supplier listed above.
- Finntip® Multistepper® pipet tips for use with single-channel pipets. These are available from Thermo Electron Corporation: www.thermo.com .


QIAprep Spin Procedure
in microcentrifuges
on vacuum manifolds
Pelleted bacteria
Resuspend
Lyse
Neutralize
Bind
Wash
Elute
Pure plasmid DNA
Bind
Vacuum
Wash
Vacuum
Elute
Pure plasmid DNA

Protocol: QIAprep Spin Miniprep Kit Using a Microcentrifuge

This protocol is designed for purification of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of *E. coli* in LB (Luria-Bertani) medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on page 37.

Please read "Important Notes" on pages 19–21 before starting.

Note: All protocol steps should be carried out at room temperature.

Procedure

1. **Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.**

   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.

2. **Add 250 µl Buffer P2 and gently invert the tube 4–6 times to mix.**

   Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

3. **Add 350 µl Buffer N3 and invert the tube immediately but gently 4–6 times.**

   To avoid localized precipitation, mix the solution gently but thoroughly, immediately after addition of Buffer N3. The solution should become cloudy.

4. **Centrifuge for 10 min at 13,000 rpm (~17,900 x *g*) in a table-top microcentrifuge.**

   A compact white pellet will form.

5. **Apply the supernatants from step 4 to the QIAprep spin column by decanting or pipetting.**

6. **Centrifuge for 30–60 s. Discard the flow-through.**

7. **(Optional): Wash the QIAprep spin column by adding 0.5 ml Buffer PB and centrifuging for 30–60 s. Discard the flow-through.**

   This step is necessary to remove trace nuclease activity when using *endA*$^+$ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α™ do not require this additional wash step.

8. **Wash QIAprep spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60 s.**

9. **Discard the flow-through, and centrifuge for an additional 1 min to remove residual wash buffer.**

   IMPORTANT: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

10. **Place the QIAprep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.**

Protocol: QIAprep Spin Miniprep Kit Using 5 ml Collection Tubes

The QIAprep Spin Miniprep procedure can be performed using 5 ml centrifuge tubes (e.g., Greiner, cat. no. 115101 or 115261) as collection tubes to decrease handling. The standard protocol on pages 23–24 should be followed with the following modifications:

Step 4: Place a QIAprep spin column in a 5 ml centrifuge tube instead of a 2 ml collection tube.

Step 6: Centrifuge at 3000 x *g* for 1 min using a suitable rotor (e.g., Beckman® GS-6KR centrifuge at ~4000 rpm). (The flow-through does not need to be discarded.)

Steps 7 and 8: For washing steps, centrifugation should be performed at 3000 x *g* for 1 min. (The flow-through does not need to be discarded.)

Step 9: Transfer the QIAprep spin column to a microcentrifuge tube. Centrifuge at maximum speed for 1 min. Continue with step 10 of the protocol.

Protocol: QIAprep Spin Miniprep Kit Using a Vacuum Manifold

This protocol is designed for purification of up to 20 µg high-copy plasmid DNA from 1–5 ml overnight cultures of *E. coli* grown in LB (Luria-Bertani) medium, using QIAprep spin columns on QIAvac 24, QIAvac 6S, or other vacuum manifolds with luer connectors. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on page 37.

Please read "Important Notes" on pages 19–21 before starting.

Note: All protocol steps should be carried out at room temperature.

Procedure

1. **Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.**

   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.

2. **Add 250 µl Buffer P2 and invert the tube gently 4–6 times to mix.**

   Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

3. **Add 350 µl Buffer N3 and invert the tube immediately but gently 4–6 times.**

   To avoid localized precipitation, immediately after addition of Buffer N3 mix the solution gently but thoroughly. The solution should become cloudy.

4. **Centrifuge for 10 min at 13,000 rpm (~17,900 x *g*) in a table-top microcentrifuge.**

   A compact white pellet will form.

   During centrifugation, prepare the vacuum manifold and QIAprep spin columns:

   QIAvac 24 (see page 16 and 18):

   - Place the QIAvac 24 lid on top of the QIAvac 24 base. Make sure that the gasket fits tightly in the groove of the QIAvac 24 lid.
   - Insert up to 24 QIAprep spin columns into the luer extensions of the QIAvac 24. Close unused positions with luer caps and connect QIAvac 24 to a vacuum source.

QIAvac 6S manifold:

(**Note**: The following procedure applies to the manifold with a hinged lid and spring lock. See pages 17 and 18.)

- Open QIAvac 6S lid. Place QIAvac luer adapter(s), or blanks (provided with QIAvac 6S) to seal unused slots, into the slots of the QIAvac top plate. Close the QIAvac 6S lid. Place the waste tray inside the QIAvac base, and place the top plate squarely over the base. Attach the QIAvac 6S to a vacuum source.
- Insert each QIAprep spin column into a luer connector on the luer adapter(s) in the vacuum manifold. Seal unused luer connectors with plugs provided with the QIAvac Luer Adapter Set.

Other vacuum manifolds: Follow the supplier's instructions. Insert each QIAprep column into a luer connector.

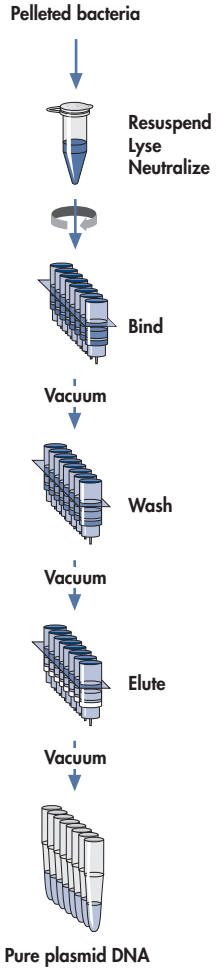
5. **Apply the supernatant from step 4 to the QIAprep spin column by decanting or pipetting.**
6. **Switch on vacuum source to draw the solution through the QIAprep spin columns, and then switch off vacuum source.**

   If using the QIAvac 24 it may be necessary to press down on the lid after the vacuum is switched on in order to achieve a tight seal.
7. **(Optional): Wash the QIAprep spin column by adding 0.5 ml Buffer PB. Switch on vacuum source. After the solution has moved through the column, switch off vacuum source.**

   This step is necessary to remove trace nuclease activity when using *endA*$^+$ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α do not require this additional wash step.
8. **Wash the QIAprep spin column by adding 0.75 ml Buffer PE. Switch on vacuum source to draw the wash solution through the column, and then switch off vacuum source.**
9. **Transfer the QIAprep spin columns to a microcentrifuge tube. Centrifuge for 1 min.**

   IMPORTANT: This extra spin is necessary to remove residual Buffer PE. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.
10. **Place the QIAprep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.**

QIAprep 8 Procedure



Protocol: QIAprep 8 Miniprep Kit

This protocol is designed for purification of high-copy plasmid DNA from up to 48 samples in parallel. Up to 20 µg DNA can be purified from 1–5 ml cultures of *E. coli* grown in LB (Luria-Bertani) medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on page 37.

Please read "Important Notes for QIAprep Procedures" on pages 19–21 before starting.

Note: All protocol steps should be carried out at room temperature.

Procedure

1. **Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.**

   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.

2. **Add 250 µl Buffer P2 and gently invert the tube 4–6 times to mix.**

   Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow lysis reaction to proceed for more than 5 min.

3. **Add 350 µl Buffer N3 and invert the tube immediately but gently 4–6 times.**

   To avoid localized precipitation, mix the solution gently but thoroughly, immediately after addition of Buffer N3. The solution should become cloudy.

4. **Centrifuge for 10 min at 13,000 rpm (~17,900 x *g*) in a table-top microcentrifuge.**

   A compact white pellet will form.

   During centrifugation, prepare QIAvac 6S:

   (**Note**: The following procedure applies to the manifold with a hinged lid and spring lock. See pages 17 and 18).

   - Open the QIAvac 6S lid and place QIAprep 8 strips in the slots of the QIAvac top plate, making sure the strips are positioned tightly. Seal any unused slots with blanks provided with the QIAvac 6S, and close the QIAvac 6S lid.
   - Place the waste tray inside the QIAvac base. Place the top plate squarely over the base. Seal any unused wells of the QIAprep strips with strip caps provided. Attach the QIAvac 6S to a vacuum source.

5. **Apply the supernatants from step 4 to the wells of the QIAprep 8 strips and switch on vacuum source.**

   Make sure the QIAvac 6S is assembled correctly before loading. Load the supernatants promptly onto the QIAprep 8 strips. If the supernatants become cloudy,

centrifuge again immediately before loading to prevent clogging the QIAprep 8 strips. The flow-through is collected in the waste tray.

6. **(Optional): Switch off vacuum and wash QIAprep 8 strips by adding 1 ml Buffer PB to each well and applying vacuum.**

   This step is recommended to remove trace nuclease activity when using *endA*$^+$ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α do not require this additional step.

7. **Switch off vacuum. Wash QIAprep 8 strips by adding 1 ml Buffer PE to each well and applying vacuum.**

   Allow Buffer PE to flow through the QIAprep 8 strips.

8. **Repeat step 7.**

9. **After Buffer PE has been drawn through all wells, apply maximum vacuum for an additional 5 min to dry the membrane.**

   IMPORTANT: This step removes residual Buffer PE from the membrane. The removal is only effective when maximum vacuum is used (i.e., turn off vacuum regulator or leakage valves if they are used), allowing maximum airflow to go through the wells.

10. **Switch off the vacuum source and ventilate the QIAvac manifold. Lift the top plate from the base (not the QIAprep strips from the top plate), vigorously tap the top plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the QIAprep strips with clean absorbent paper. Proceed to step 11a or 11b.**

    This step removes residual Buffer PE, which may be present around the outlet nozzles and collars of the QIAprep 8 strips. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

11a. **For elution into collection microtubes provided:**

    Replace waste tray with the blue collection microtube rack (provided with the QIAvac 6S) containing 1.2 ml collection microtubes. Place the top plate back on the base.

    Rows of collection microtubes should be lined up with the QIAprep 8 strips.

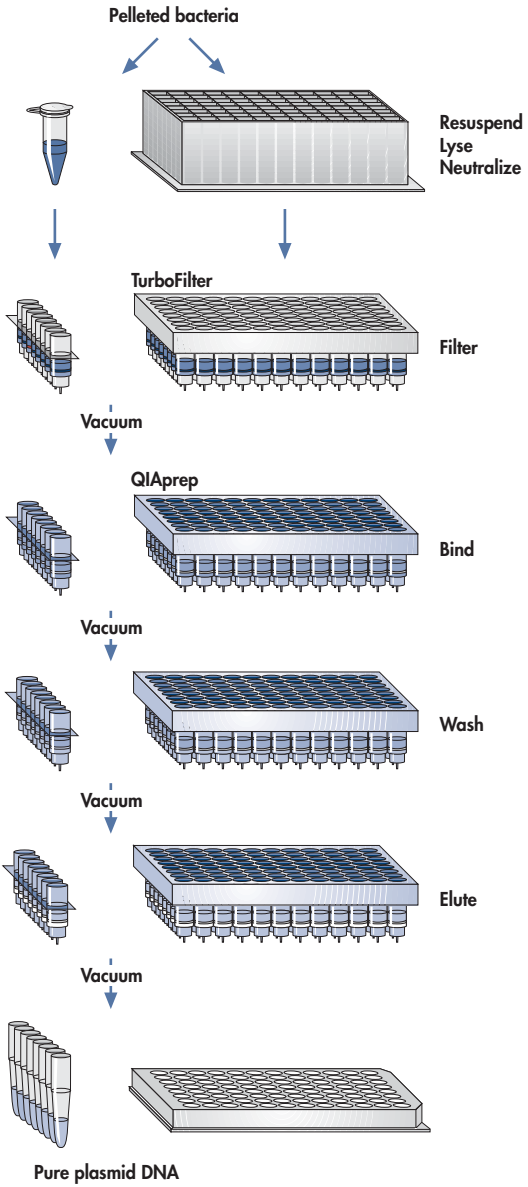
11b. **For elution into a 96-well microtiter plate:**

    Replace waste tray with empty blue collection microtube rack (provided with QIAvac 6S) and place a 96-well microtiter plate directly on the rack. Place the top plate back on the base.

12. **Elute DNA by adding 100 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each well of the QIAprep 8 Strips, let stand for 1 min, and switch on vacuum source. After elution, switch off vacuum source and ventilate the QIAvac 6S slowly.**

    For increased DNA concentration, an elution volume of 75 µl can be used.

QIAprep 8 and 96 Turbo Procedure



Protocol: QIAprep 8 Turbo Miniprep Kit

This protocol is designed for medium-throughput plasmid DNA minipreps using TurboFilter 8 and QIAprep 8 strips on QIAvac 6S. The kit accommodates 8–48 parallel preparations of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of *E. coli* grown in LB (Luria-Bertani) medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on page 37. DNA purification can be automated, please call QIAGEN for more details.

Please read "Important Notes" on pages 19–21 before starting.

Note: All protocol steps should be carried out at room temperature.

Procedure

1. **Resuspend pelleted bacterial cells in 250 µl Buffer P1.**

   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.

2. **Add 250 µl Buffer P2 to each sample, gently invert the tubes 4–6 times to mix, and incubate at room temperature for 5 min.**

   It is important to mix gently by inverting the tubes. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tubes until the solution becomes viscous and slightly clear.

   During incubation prepare the QIAvac 6S (see pages 17 and 18):

   - Open QIAvac 6S lid and place TurboFilter 8 Strips in the slots of the QIAvac top plate. Make sure the strips are positioned tightly. Seal any unused slots with blanks (provided with QIAvac 6S), and close QIAvac 6S lid.
   - Place the strip holder inside the QIAvac base. Place QIAprep 8 strips into the strip holder such that a QIAprep strip is placed under each TurboFilter strip.
   - Place the top plate squarely over the base. Seal any unused wells of the TurboFilter strips with the strip caps provided. Attach the QIAvac to vacuum source.

3. **Add 350 µl Buffer N3 to each sample and invert the tubes immediately but gently 4–6 times.**

   To avoid localized precipitation, mix the samples gently but thoroughly, immediately after addition of Buffer N3. The solutions should become cloudy.

4. **Pipet the lysates from step 3 (850 µl per well) into the wells of the TurboFilter strips. Unused wells of TurboFilter strips should be sealed with the strip caps provided. Apply vacuum until all samples have passed through TurboFilter.**

   Optimal flow rate is 1–2 drops/s, which can be regulated by using a 3-way valve or vacuum regulator (see page 47) between the QIAvac and the vacuum source.

5. **Switch off vacuum, and ventilate the QIAvac 6S slowly. Discard the TurboFilter strips. Transfer the QIAprep strips containing the cleared lysates into the slots of the QIAvac top plate. Close QIAvac 6S lid. Replace strip holder in the base with the waste tray. Place the top plate squarely over the base. Seal any unused wells of the QIAprep strips with the strip caps provided. Apply vacuum.**

   The flow-through is collected in the waste tray.

6. **(Optional): Switch off vacuum, and wash QIAprep strips by adding 1 ml Buffer PB to each well and applying vacuum.**

   This step is necessary to remove trace nuclease activity when using *endA*$^+$ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α do not require this additional step.

7. **Switch off vacuum. Wash QIAprep strips by adding 1 ml Buffer PE to each well and applying vacuum. Repeat once.**

8. **After Buffer PE has been drawn through all wells, apply maximum vacuum for an additional 5 min to dry the membrane.**

   IMPORTANT: This step removes residual Buffer PE from the membrane. The removal is only effective when maximum vacuum is used (i.e., turn off vacuum regulator or leakage valves if they are used), allowing maximum airflow to go through the wells.

9. **Switch off vacuum, and ventilate the QIAvac 6S slowly. Lift the top plate from the base (not the QIAprep strips from the top plate), vigorously tap the top plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the QIAprep strips with clean absorbent paper. Proceed either to step 10a or 10b, as desired.**

   This step removes residual Buffer PE, which may be present around the outlet nozzles and collars of QIAprep strips. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

10a. **For elution into collection microtubes provided:**

   Replace waste tray with the blue collection microtube rack (provided with the QIAvac 6S) containing 1.2 ml collection microtubes. Place the top plate back on the base.

   Rows of collection microtubes should be lined up with the QIAprep 8 strips.

10b. **For elution into a 96-well microtiter plate:**

   Replace waste tray with empty blue collection microtube rack (provided with the QIAvac 6S) and place a 96-well microtiter plate directly on the rack. Place the top plate back on the base.

11. **To elute DNA, add 100 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each well of the QIAprep strips, let stand 1 min, and apply maximum vacuum for 5 min. Switch off vacuum and ventilate the QIAvac 6S slowly.**

   For increased DNA concentration, an elution volume of 75 µl can be used.

Protocol: QIAprep 96 Turbo Miniprep Kit

This protocol is designed for high-throughput plasmid DNA minipreps using TurboFilter 96 and QIAprep 96 plates on QIAvac 96. The kit accommodates up to 96 parallel preparations of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of *E. coli* grown in LB (Luria-Bertani) medium. If 1.3 ml overnight cultures are used, up to 96 cultures can be grown in a flat-bottom block (see page 36 for protocol). For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on page 37. DNA purification can be automated, please call QIAGEN for more details.

Please read "Important Notes" on pages 19–21 before starting.

Note: All protocol steps should be carried out at room temperature.

Procedure

1. **Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to the flat-bottom block provided with the kit.**

   Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.

2. **Add 250 µl Buffer P2 to each sample, seal the block with the tape provided, gently invert the block 4–6 times to mix, and incubate at room temperature for 5 min.**

   It is important to mix gently by inverting the block. Do not shake vigorously, as this will result in shearing of genomic DNA. If necessary, continue inverting the block until the solution becomes viscous and slightly clear.

   During incubation prepare QIAvac 96 (see pages 17 and 18):

   - Place the TurboFilter 96 plate in the QIAvac top plate, make sure that the plate is seated securely. Seal unused wells of the TurboFilter with tape.
   - Place the plate holder inside the QIAvac base. Place QIAprep 96 plate into the plate holder.
   - Place QIAvac 96 top plate squarely over base. The QIAprep plate should now be positioned under the TurboFilter plate. Attach QIAvac to a vacuum source.

3. **Remove the tape from the block. Add 350 µl Buffer N3 to each sample and seal the block with a new tape sheet. Gently invert the block 4–6 times.**

   To avoid localized precipitation, mix the samples gently but thoroughly, immediately after addition of Buffer N3. The solutions should become cloudy.

4. **Remove the tape from the block. Pipet the lysates from step 3 (850 µl per well) into the wells of the TurboFilter plate. Unused wells of the TurboFilter plate should be sealed with tape. Apply vacuum until all samples have passed through.**

   The optimal flow rate is approximately 1–2 drops/s, which can be regulated by using a 3-way valve or vacuum regulator (see page 47) between the QIAvac and the vacuum source.

5. **Switch off vacuum and ventilate the QIAvac 96 slowly. Discard the TurboFilter plate. Transfer the QIAprep plate containing the cleared lysates to the top plate of the manifold. Seal any unused wells of the QIAprep plate with tape. Replace plate holder in the base with waste tray. Place the top plate squarely over the base, making sure that the QIAprep plate is seated securely. Apply vacuum.**

   The flow-through is collected in the waste tray.

6. **(Optional): Switch off vacuum, and wash QIAprep plate by adding 0.9 ml Buffer PB to each well and applying vacuum.**

   This step is necessary to remove trace nuclease activity when using *endA*$^+$ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5α do not require this additional step.

7. **Switch off vacuum. Wash QIAprep plate by adding 0.9 ml of Buffer PE to each well and applying vacuum. Repeat once.**

8. **After Buffer PE has been drawn through all wells, apply maximum vacuum for an additional 10 min to dry the membrane.**

   IMPORTANT: This step removes residual Buffer PE from the membrane. The removal is only effective when maximum vacuum is used (i.e., turn off vacuum regulator or leakage valves if they are used), allowing maximum airflow to go through the wells.

9. **Switch off vacuum, and ventilate the QIAvac 96 slowly. Lift the top plate from the base (not the QIAprep plate from the top plate), vigorously tap the top plate on a stack of absorbent paper until no drops come out, and blot the nozzles of the QIAprep plate with clean absorbent paper. Proceed either to step 10a, or 10b, as desired.**

   This step removes residual Buffer PE, which may be present around the outlet nozzles and collars of QIAprep plate. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

10a. For elution into provided collection microtubes:

Replace waste tray with the blue collection microtube rack containing 1.2 ml collection microtubes. Place the top plate back on the base, making sure that the QIAprep plate is seated securely.

10b. For elution into a 96-well microtiter plate:

Replace waste tray with an empty blue collection microtube rack. Place a 96-well microtiter plate directly on the rack. Place the top plate back on the base, making sure that the QIAprep plate is positioned securely.

11. To elute DNA, add 100 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each well of the QIAprep plate, let stand for 1 min, and apply maximum vacuum for 5 min. Switch off vacuum and ventilate QIAvac 96 slowly.

For increased DNA concentration, an elution volume of 75 µl can be used.

Protocol: Cell Cultivation in a 96-Well Block

1. **Fill each well of a 96-well flat-bottom block with 1.3 ml of growth medium containing the appropriate selective agent. Inoculate each well from a single bacterial colony. Incubate the cultures for 20–24 h at 37°C with shaking at 300 rpm.**

   The wells in the block may be protected against spill-over by covering the block with a plastic lid or adhesive tape. AirPore microporous tape sheets promote gas exchange during culturing (see ordering information, page 46). If non-porous tape is used, pierce 2–3 holes in the tape with a needle above each well for aeration.

2. Harvest the bacterial cells in the block by centrifugation for 5 min at 1500 x *g* in a centrifuge with a rotor for microtiter plates (e.g., Sigma® 6K10, or Heraeus Minifuge® GL). The block should be covered with adhesive tape during centrifugation. Remove media by inverting the block.

   To remove the media, peel off the tape and quickly invert the block over a waste container. Tap the inverted block firmly on a paper towel to remove any remaining droplets of medium.

   WARNING: Ensure that the buckets on the rotor have sufficient clearance to accommodate the 2 ml flat-bottom blocks before starting the centrifuge.

3. **Resuspend each bacterial pellet in 250 µl Buffer P1, seal the block with a tape sheet* and resuspend the pellets by vortexing.**

   No cell clumps should be visible after cell resuspension.

4. **Remove the tape. Proceed with step 2 of the QIAprep 96 Turbo Miniprep Kit protocol on page 33.**

* Provided with QIAprep 96 Turbo Miniprep Kits.

Special Applications

Purification of low-copy plasmids and cosmids

All QIAprep miniprep protocols in this handbook can be used for preparation of low-copy-number plasmid or cosmids from 1–10 ml overnight *E. coli* cultures grown in LB medium.

Only two slight modifications to the protocols are required:

- The wash step with Buffer PB is required for all strains.
- When plasmid or cosmids are >10 kb, pre-heat Buffer EB (or water) to 70°C prior to eluting DNA from the QIAprep membrane. A 10 ml overnight LB culture typically yields 5–10 µg DNA.

Note: When using 10 ml culture volume, it is recommended to double the volumes of Buffers P1, P2, and N3 used.

Purification of very large plasmids (>50 kb)

Plasmids >50 kb elute less efficiently from silica than smaller plasmids, but do elute efficiently from QIAGEN anion-exchange resin. QIAGEN provides the anion-exchange-based QIAGEN Large-Construct Kit for efficient large-scale purification of ultrapure genomic DNA-free BAC, PAC, P1, or cosmid DNA. For high-throughput, small-scale purification of BACs, PACs, and P1s, an optimized alkaline lysis protocol in R.E.A.L.® Prep 96 Kits yields DNA suitable for sequencing and screening. Call QIAGEN Technical Services or your local distributor for more information on these kits, or see ordering information on page 47.

Purification of plasmid DNA prepared by other methods

Plasmid DNA isolated by other methods can be further purified using QIAprep modules and any of the QIAprep protocols in this handbook.

1. Add 5 volumes of Buffer PB to 1 volume of the DNA solution and mix (e.g., add 500 µl Buffer PB to 100 µl of DNA sample).
2. Apply the samples to QIAprep spin columns or to the wells of a QIAprep 8 strip or 96-well plate. Draw the samples through the QIAprep membrane by centrifugation or vacuum, and continue the appropriate protocol at the Buffer PE wash step. The optional wash step with Buffer PB is not necessary.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol(s) in this handbook or molecular biology applications (see back cover for contact information).

		Comments and suggestions
Low or no yield		
General		Low yields may be caused by a number of factors. To find the source of the problem, analyze fractions saved from each step in the procedure on an agarose gel (e.g., Figure 6, page 45). A small amount of the cleared lysate and the entire flow-through can be precipitated by adding 0.7 volumes isopropanol and centrifuging at maximum speed (13,000 rpm or ~17,000 x *g*) for 30 minutes. The entire wash flow-through can be precipitated by adding 0.1 volumes of 3 M sodium acetate, pH 5.0, and 0.7 volumes of isopropanol.
No DNA in the cleared lysate before loading		
a)	Plasmid did not propagate	Read "Growth of bacterial cultures" (pages 41–43) and check that the conditions for optimal growth were met.
b)	Lysate prepared incorrectly	Check storage conditions and age of buffers.
c)	Buffer P2 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 flow-through of cleared lysate		
a)	QIAprep membrane overloaded	If rich culture media, such as TB or 2x YT are used, culture volumes must be reduced. It may be necessary to adjust LB culture volume if the plasmid and host strain show extremely high copy number or growth rates. See "Culture media" on page 43.

b)	RNase A digestion omitted	Ensure that RNase A is added to Buffer P1 before use.
c)	RNase A digestion insufficient	Reduce culture volume if necessary. If Buffer P1 containing RNase A is more than 6 months old, add additional RNase A.

DNA is found in the wash flow-through

a)	Ethanol omitted from wash buffer	Repeat procedure with correctly prepared wash buffer (Buffer PE).

Little or no DNA in eluate

a)	Elution buffer incorrect	DNA is eluted only in the presence of low-salt buffer, e.g., Buffer EB (10 mM Tris·Cl, pH 8.5) or water. Elution efficiency is dependent on pH. The maximum efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH value is within this range.
b)	Elution buffer incorrectly dispensed onto membrane	Add elution buffer to the center of the QIAprep membrane to ensure that the buffer completely covers the surface of the membrane for maximum elution efficiency.

Low DNA quality

DNA does not perform well

a)	Eluate salt concentration too high	For the QIAprep spin column, modify the wash step by incubating the column for 5 minutes at room temperature after adding 0.75 ml of Buffer PE and then centrifuging. For QIAprep 8 preparations and QIAprep 8 and 96 Turbo preparations, ensure that two wash steps are carried out prior to elution.
b)	Nuclease contamination	When using *endA*$^+$ host strains such as HB101 and its derivatives, the JM series, or any wild-type strain, ensure that the wash step with Buffer PB is performed.
c)	Eluate contains residual ethanol	Ensure that step 9 in the QIAprep Spin Miniprep protocol and steps 9 and 10 in the QIAprep 8 Miniprep, QIAprep 8 Turbo Miniprep, or QIAprep 96 Turbo Miniprep protocols are performed.

RNA in the eluate

a)	RNase A digestion omitted	Ensure that RNase A is added to Buffer P1 before use.
b)	RNase A digestion insufficient	Reduce culture volume if necessary. If Buffer P1 containing RNase A is more than 6 months old, add additional RNase A.

Genomic DNA in the eluate

a)	Buffer P2 added incorrectly	The lysate must be handled gently after addition of Buffer P2 to prevent shearing. Reduce culture volume if lysate is too viscous for gentle mixing.
b)	Buffer N3 added incorrectly	Upon addition of Buffer N3 in step 3, mix immediately but gently.
c)	Lysis too long	Lysis in step 2 must not exceed 5 minutes.
d)	Culture overgrown	Overgrown cultures contain lysed cells and degraded DNA. Do not grow cultures for longer than 12–16 hours.

Appendix A: Background Information

Growth of bacterial cultures

Plasmids are generally prepared from bacterial cultures grown in the presence of a selective agent such as an antibiotic (3,4). The yield and quality of plasmid DNA may depend on factors such as plasmid copy number, host strain, inoculation, antibiotic, and type of culture medium.

Plasmid copy number

Plasmids vary widely in their copy number per cell (Table 6), depending on their origin of replication (e.g., pMB1, ColE1, or pSC101) which determines whether they are under relaxed or stringent control; and depending on the size of the plasmid and its associated insert. Some plasmids, such as the pUC series and derivatives, have mutations which allow them to reach very high copy numbers within the bacterial cell. Plasmids based on pBR322 and cosmids are generally present in lower copy numbers. Very large plasmids and cosmids are often maintained at very low copy numbers per cell.

Table 6. Origins of replication and copy numbers of various plasmids (3).

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	pMB1*	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-number plasmids listed here contain mutated versions of this origin.

Host strains

Most *E. coli* strains can be used successfully to isolate plasmid DNA, although the strain used to propagate a plasmid has an effect on the quality of the purified DNA. Host strains such as DH1, DH5α, and C600 give high-quality DNA. The slower growing strain XL1-Blue also yields DNA of very high-quality which works extremely well for sequencing. Strain HB101 and its derivatives, such as TG1 and the JM series, produce large amounts of carbohydrates which are released during lysis and can inhibit enzyme activities if not completely removed (4). In addition, these strains have high levels of endonuclease activity which can reduce DNA quality. The methylation and growth characteristics of the strain should also be taken into account when selecting a host strain. XL1-Blue and DH5α are highly recommended for reproducible and reliable results.

Inoculation

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

The desired clone should be streaked from a glycerol stock onto a freshly prepared agar plate containing the appropriate selective agent so that single colonies can be isolated. This procedure should then be repeated to ensure that a single colony of an antibiotic-resistant clone can be picked. A single colony should be inoculated into 1–5 ml of media containing the appropriate selective agent, and grown with vigorous shaking for 12–16 hours. Growth for more than 16 hours is not recommended since cells begin to lyse and plasmid yields may be reduced.

Antibiotics

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

The stability of the selective agent should also be taken into account. Resistance to ampicillin, for example, is mediated by β-lactamase which is encoded by the plasmid-linked *bla* gene and which hydrolyzes ampicillin. Levels of ampicillin in the culture medium are thus continually depleted. This phenomenon is clearly demonstrated on ampicillin plates, where "satellite colonies" appear as the ampicillin is hydrolyzed in the vicinity of a growing colony. Ampicillin is also very sensitive to temperature, and when in solution should be stored frozen in single-use aliquots. The recommendations given in Table 7 are based on these considerations.

Table 7. Concentrations of Commonly Used Antibiotics

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

Culture media

Luria-Bertani (LB) broth is the recommended culture medium for use with QIAprep Kits, since richer broths such as TB (Terrific Broth) or 2x YT lead to extremely high cell densities, which can overload the purification system. It should be noted that cultures grown in TB may yield 2–5 times the number of cells compared to cultures grown in LB broth. If these media are used, recommended culture volumes must be reduced to match the capacity of the QIAprep membrane. If excess culture volume is used, alkaline lysis will be inefficient, the QIAprep membrane will be overloaded, and the performance of the system will be unsatisfactory. Furthermore, the excessive viscosity of the lysate will require vigorous mixing, which may result in shearing of bacterial genomic DNA and contamination of the plasmid DNA. Care must also be taken if strains are used which grow unusually fast or to very high cell densities. In such cases, doubling the volumes of Buffers P1, P2, and N3 may be beneficial. It is best to calculate culture cell density and adjust the volume accordingly.

Please note that a number of slightly different LB culture broths, containing different concentrations of NaCl, are in common use. Although different LB broths produce similar cell densities after overnight culture, plasmid yields can vary significantly. We recommend growing cultures in LB medium containing 10 g NaCl, 10 g tryptone, and 5 g yeast extract per liter to obtain the highest plasmid yields with the QIAprep system.

Preparation of cell lysates

Bacteria are lysed under alkaline conditions. After harvesting and resuspension, the bacterial cells are lysed in NaOH/SDS (Buffer P2) in the presence of RNase A (2, 5). SDS solubilizes the phospholipid and protein components of the cell membrane, leading to lysis and release of the cell contents while the alkaline conditions denature the chromosomal and plasmid DNAs, as well as proteins. The optimized lysis time allows maximum release of plasmid DNA without release of chromosomal DNA, while minimizing the exposure of the plasmid to denaturing conditions. Long exposure to alkaline conditions may cause the plasmid to become irreversibly denatured (2). This denatured form of the plasmid runs faster on agarose gels and is resistant to restriction enzyme digestion.

The lysate is neutralized and adjusted to high-salt binding conditions in one step by the addition of Buffer N3. The high salt concentration causes denatured proteins, chromosomal DNA, cellular debris, and SDS to precipitate, while the smaller plasmid DNA renatures correctly and stays in solution. It is important that the solution is thoroughly and gently mixed to ensure complete precipitation.

To prevent contamination of plasmid DNA with chromosomal DNA, vigorous stirring and vortexing must be avoided during lysis. Separation of plasmid from chromosomal DNA is based on coprecipitation of the cell wall-bound chromosomal DNA with insoluble complexes containing salt, detergent, and protein. Plasmid DNA remains in the clear supernatant. Vigorous treatment during the lysis procedure will shear the bacterial chromosome, leaving free chromosomal DNA fragments in the supernatant. Since chromosomal fragments are chemically indistinguishable from plasmid DNA under the conditions used, the two species will not be separated on QIAprep membrane and will elute under the same low-salt conditions. Mixing during the lysis procedure must therefore be carried out by slow, gentle inversion of the tube.

Appendix B: Agarose Gel Analysis of Plasmid DNA

The QIAprep Miniprep procedure can be analyzed using agarose gel electrophoresis as shown in Figure 6. Samples can be taken from the cleared lysate and its flow-through, precipitated with isopropanol and resuspended in a minimal volume of TE buffer. In Figure 6 the cleared lysate shows closed circular plasmid DNA and degraded RNase A-resistant RNA. The flow-through contains only degraded RNA and no plasmid DNA is present. The eluted pure plasmid DNA shows no contamination with other nucleic acids.

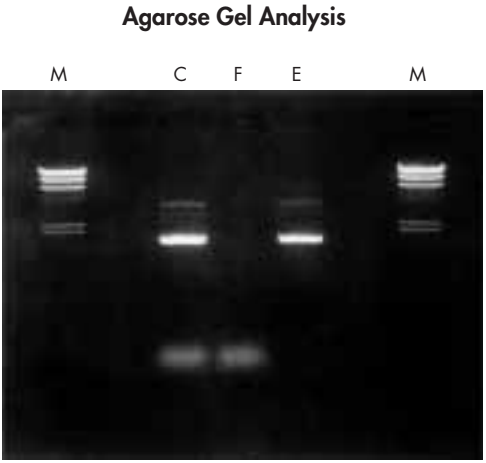


Figure 6 Agarose gel analysis of the QIAprep Miniprep procedure. **C**: cleared lysate; **F**: flow-through; **E**: eluted plasmid; **M**: markers.

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QIAprep 8 Turbo BioRobot Kit (48)	For 48 x 8 plasmid minipreps, 48 each: TurboFilter 8 and QIAprep 8 Strips, Reagents, Buffers, Collection Microtubes (1.2 ml), Caps, 96-Well Microplates RB and Lids	962134
QIAprep 96 Turbo Miniprep Kit (4)†	For 4 x 96 plasmid minipreps: 4 TurboFilter 96 Plates, 4 QIAprep 96 Plates, 4 Flat-Bottom Blocks with Lids, Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	27191
QIAprep 96 Turbo Miniprep Kit (24)†	For 24 x 96 Plasmid minipreps: 24 x TurboFilter 96 Plates, 24 x QIAprep 96 Plates, 24 Flat-Bottom Blocks with Lids, Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	27193

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QIAvac 96	Vacuum manifold for processing QIAGEN 96-well plates: includes QIAvac 96 Top Plate, Base, Waste Tray, Plate Holder	19504
QIAvac Luer Adapter Set‡	For processing 1–24 QIAprep Spin Columns: 6 adapters, each with 4 luer connectors, 24 plugs	19541
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* Requires the use of QIAvac 6S. † Requires the use of QIAvac 96. ‡ Compatible only with QIAvac Top Plates containing flip-up lid. § ATP solution required for exonuclease digestion is not provided.

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