New Edition

QIAwell® System Handbook

For

QIAwell 8 Ultra Plasmid Kit

QIAwell 96 Ultra Plasmid Kit

August 2001

QIAGEN

QIAGEN Worldwide

QIAGEN Companies

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www.qiagen.com

QIAGEN Distributors

Please see the last page for contact information for your local QIAGEN distributor.

Contents

Kit Contents

QIAwell 8 Ultra Plasmid Kit	**(10)**
Catalog No.	**16152**
No. of Preparations	**80**
QIAfilter™ 8 Strips	10
QIAwell 8 Strips	10
QIAprep® 8 Strips	10
Buffer P1	50 ml
Buffer P2	50 ml
Buffer P3	50 ml
Buffer QW	260 ml
Buffer QE	130 ml
Buffer PE (concentrate)	50 ml
Buffer EB	60 ml
RNase A*	5 mg
Collection Microtubes	80
Collection Microtube Caps	80
Strip Caps	40
Handbook	1

QIAwell 96 Ultra Plasmid Kit	**(4)**
Catalog No.	**16191**
No. of Preparations	**384**
QIAfilter 96 Plates	4
QIAwell 96 Plates	4
QIAprep 96 Plates	4
Buffer P1	150 ml
Buffer P2	150 ml
Buffer P3	150 ml
Buffer QW	1000 ml
Buffer QE	450 ml
Buffer PE (concentrate)	200 ml
Buffer EB	120 ml
RNase A*	15 mg
Collection Microtubes	384
Collection Microtube Caps	384
Strip Caps	200
Handbook	1

** Provided as a 100 mg/ml solution*

Storage Conditions

QIAfilter, QIAwell, and QIAprep strips or plates should be stored dry and at room temperature. They can be stored for up to 12 months without showing any reduction in performance, capacity, or quality of separation. After addition of RNase A, Buffer P1 should be stored at 2–8°C and is stable for 6 months. RNase A stock solution can be stored for two years at room temperature.

Buffers		Storage
Buffer P1	Resuspension buffer	2–8°C (following RNase A addition)
Buffer P2	Lysis buffer	RT
Buffer P3	Neutralization buffer	2–8°C*
Buffer QW	QIAwell wash buffer	RT
Buffer QE	QIAwell elution buffer	RT
Buffer PE	QIAprep wash buffer	RT
Buffer EB	QIAprep elution buffer	RT
RNase A	Stock solution	RT

** Buffer P3 is stored at 2–8°C for convenience, to save pre-chilling before use.*

Quality Control

As part of the stringent QIAGEN quality assurance program, the performance of QIAwell Ultra Plasmid Kits is monitored routinely on a lot-to-lot basis. All components are tested separately to ensure highest performance and reliability.

Product Use Limitations

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

Product Warranty and Satisfaction Guarantee

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

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided

on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see inside front 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. They are always available to discuss any general or specific questions you may have. If you have any questions or experience any problems regarding QIAwell Ultra Plasmid Kits, or QIAGEN products in general, please do not hesitate to contact us.

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

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see inside front 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 this kit and its components.

The following risk and safety phrases apply to components of the QIAwell 8 Ultra Plasmid Kit and the QIAwell 96 Ultra Plasmid Kit.

Buffer P2

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

Buffer P3

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

Buffer QE

Contains sodium perchlorate: harmful. Risk and safety phrases*: R22, S13-26-36-46

RNase A

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

** 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 protective clothing and gloves; S46: If swallowed, seek medical advice immediately and show this container or label.*

Introduction

The QIAwell Plasmid Purification System provides maximum convenience for plasmid purification from moderate to large numbers of samples. This product is the result of collaboration between researchers at QIAGEN and 3M, and the ongoing commitment at QIAGEN to simplify and improve DNA template preparation. Plasmid DNA prepared using the QIAwell System is suitable for the most demanding applications, including fluorescent DNA sequencing and high-throughput transfection. The QIAwell System rapidly provides up to 96 ultrapure, double-stranded plasmid DNA templates with minimal effort.

The QIAGEN Guide to Template Purification and DNA Sequencing (2nd Edition) provides a general overview of commonly used sequencing methods and technical information on sequencing optimization and troubleshooting. This comprehensive guide is available free on request.

This handbook is a revised edition of the *QIAwell System Handbook*, November 1998. The changes are a result of ongoing research and development at QIAGEN and valuable feedback from customers.

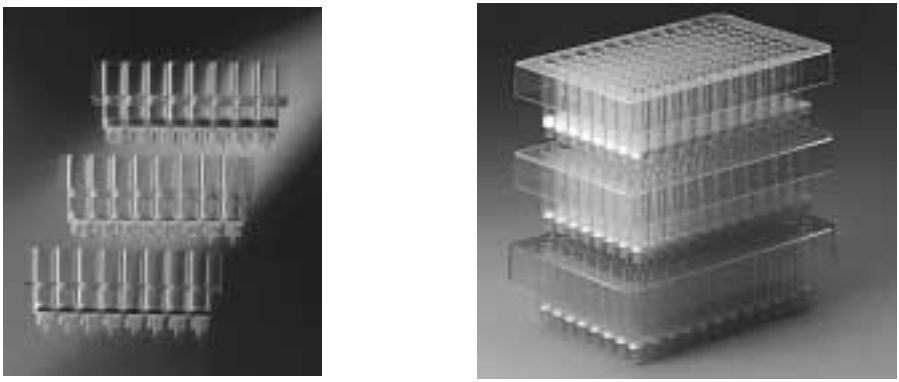


Figure 1. *Components of the QIAwell 8 Ultra System (left) and QIAwell 96 Ultra System (right)*

QIAwell System Technology

The QIAwell System consists of three independent multiwell modules (Figure 1, page 8) which can be used separately or sequentially on the vacuum manifolds QIAvac 6S and QIAvac 96 (pages 11–13). Alternatively, the procedure can also be automated on the BioRobot® 9600 and 3000 (page 14). Special kit formats optimized for use with BioRobot workstations are available. For details of QIAGEN® Anion-Exchange Resin and QIAwell purification chemistry, please refer to Appendices A, B, and C (pages 32–39).

Clearing Lysates, without centrifugation, on QIAfilter modules

QIAfilter 8 and QIAfilter 96 modules (yellow) have been developed to ensure preparation of optimally cleared lysates. Filtration of the lysate through the special asymmetric filter removes all traces of denatured and SDS-precipitated cellular constituents without time-consuming centrifugation. Cleared lysates are transferred directly into the corresponding QIAwell module using either the QIAvac 6S or QIAvac 96.

Purification on QIAwell modules

Using the QIAvac 6S or QIAvac 96 vacuum manifolds, the cleared lysates are loaded into the QIAwell module (clear) and a low to moderate vacuum gently pulls the entire lysate through the QIAwell membranes. The salt and pH conditions in the lysates ensure that only DNA will bind to the immobilized QIAGEN Resin, while degraded RNA, cellular proteins, and metabolites are not retained and are found in the flow-through fraction. The QIAwell membranes are then washed with Buffer QW, which disrupts any protein–DNA interactions, allowing the removal of nucleic acid-binding proteins and other residual contaminants from the plasmid DNA without the use of phenol.

Desalting and concentration

The purified plasmid DNA is eluted from the QIAwell membrane with Buffer QE. Desalting and concentration take place in-line in the QIAprep module (blue), which eliminates time-consuming precipitation and centrifugation. Double-stranded plasmid DNA binds to the silica-gel-based membrane of the QIAprep module; salts and other non-DNA constituents are efficiently removed by washing with Buffer PE. Pure plasmid DNA is eluted from QIAprep modules using 150 µl of Buffer EB. The DNA is suitably concentrated, e.g., 150–200 ng/µl from 5 ml LB culture (Figure 2, page 10), for use in most applications, including fluorescent cycle-sequencing reactions.

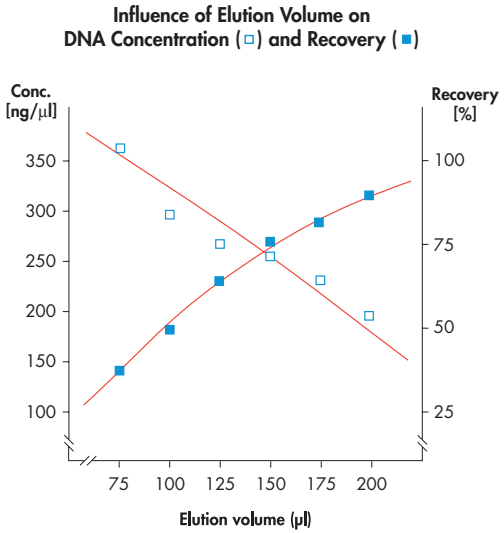


Figure 2. *Desalting on the QIAprep module. Effect of elution volumes on DNA concentration and recovery using QIAwell Ultra Plasmid Kits.*

QIAvac 6S and QIAvac 96 Vacuum Manifolds

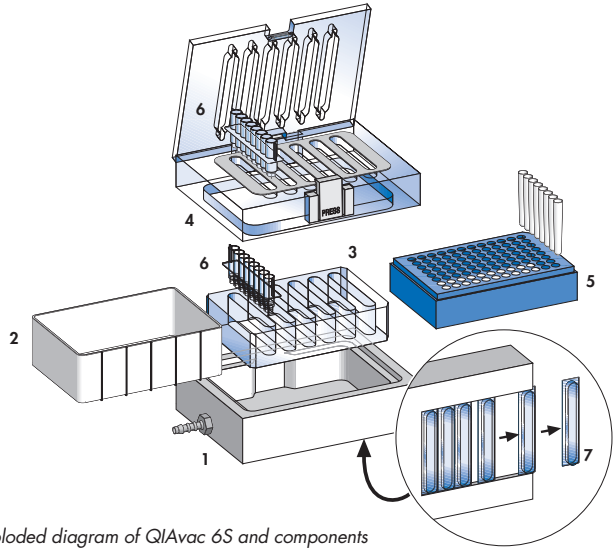


Figure 3. *Exploded diagram of QIAvac 6S and components*

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*
5. *Microtube rack*
6. *8-well strip**
7. *Blanks to seal unused slots*

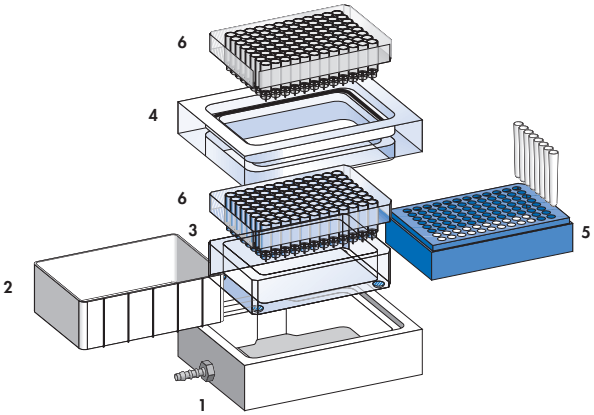


Figure 4. *Exploded diagram of QIAvac 96 and components*

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. *Disposable microtube rack**
6. *96-well plate**

**Not included with QIAvac. Included in the appropiate QIAwell Kit.*

Guidelines for QIAvac 6S and QIAvac 96

QIAvac 6S and QIAvac 96 facilitate DNA minipreparations by providing a convenient modular vacuum manifold for use with the QIAwell System. The following recommendations should be followed when handling the QIAvac 6S or QIAvac 96 vacuum manifold:

- QIAvac 6S and QIAvac 96 operate with house vacuum, vacuum pump, or water aspirator.
- Always store QIAvac 6S and QIAvac 96 vacuum 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 6S and QIAvac 96 vacuum manifolds 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 1). 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 vacuum manifold. The spring lock on the top plate and the self-sealing gasket 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 1. 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	

Recommended vacuum pressures

- Optimum vacuum is between –200 and –300 mbar for sample transfer and elution, and up to maximum vacuum for ethanol (Buffer PE) removal.
- The negative pressure (vacuum) should be assessed before beginning the procedure by applying vacuum to **empty** modules on the QIAvac manifold, as indicated in Table 2.
- 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 41). Vacuum recommendations are given in negative units (Table 2) to indicate the required reduction in pressure with respect to the atmosphere. Table 3 provides pressure conversions to other units.
- If no vacuum calibration is available, adjust vacuum so that samples drip rather than flow from one module to the next.
- Use of excessive vacuum can cause insufficient binding and sample spattering, while use of insufficient vacuum pressure may reduce DNA yield and purity.

Table 2. Regulation of vacuum pressure for QIAwell procedures

Procedure	Vacuum manifold	Module used for checking pressure*	Vacuum pressure‡ mbar	Vacuum pressure‡ mm Hg
QIAwell 8 Ultra	QIAvac 6S	QIAfilter 8 Strip(s)†	–200 to –300	–150 to –225
QIAwell 96 Ultra	QIAvac 96	QIAfilter 96 Plate	–200 to –300	–150 to –225

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 3. 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)	0.0394
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per square inch (psi)	0.0145

Automation of the QIAwell 96 procedure on BioRobot® Systems

QIAwell Ultra 96 procedures for purification of plasmid, cosmid, BAC, PAC, and P1 DNA can be automated on QIAGEN BioRobot Systems. A special kit format optimized for use with BioRobot Systems is available (see Ordering Information, page 40).

BioRobot Systems are versatile workstations designed to automate high-throughput liquid handling and sample processing. Pre-programmed protocols are available for purification of nucleic acids, reaction setup, and other liquid handling applications. Table 4 lists applications currently available for BioRobot workstations. Chemistry, hardware, and software are all supplied by QIAGEN, providing a complete automation system for molecular biology and molecular diagnostic laboratories. For detailed specifications and ordering information, call QIAGEN*.

Table 4. Applications currently available for automation on BioRobot workstations*

Application	BioRobot Kits	BioRobot workstation 9600	3000	8000
DNA cleanup	QIAquick 96 PCR BioRobot Kit†	✓	✓	✓
Ultrapure plasmid minipreps	QIAwell 96 Ultra BioRobot Kit†	✓	✓	✓
High-purity plasmid minipreps	QIAprep 8 Turbo BioRobot Kit†	✓	✓	
	QIAprep 96 Turbo BioRobot Kit†	✓	✓	✓
Standard-purity plasmid minipreps	R.E.A.L. Prep 96 BioRobot Kit†	✓	✓	✓
Protein purification	Ni-NTA Superflow 96 BioRobot Kit†	✓	✓	✓
Protein purification and assay	Ni-NTA Magnetic Agarose Beads†		✓	
Sequencing Reaction Setup		✓	✓	✓
Dye-terminator removal	DyeEx 96 Kit	✓	✓	
PCR, RT-TaqMan Reaction Setup		✓	✓	✓

** QIAGEN Robotic Systems are not available in all countries; please inquire.*

† Larger kit sizes, and special kit formats, for use with the BioRobot 8000 are also available; please inquire.

QIAwell Plasmid Purification Protocols

Important notes before starting

Please refer to Appendix B on pages 35–37 which contains useful information concerning culture growth and other parameters which can affect DNA yield from bacterial cultures.

In this handbook there are protocols corresponding to two kits:

- QIAwell 8 Ultra Plasmid Kit
- QIAwell 96 Ultra Plasmid Kit

Please ensure that the protocol selected is the one appropriate for your kit.
These protocols are designed for purification of up to 20 µg of high-copy plasmid from up to 5 ml of bacterial culture grown in Luria-Bertani (LB) medium.

The following notes apply to both protocols.

Notes

- Bacterial cultures should be grown in LB medium. If rich medium such as 2x YT or TB are used, culture volumes must be titrated to avoid yield reductions and loss of DNA quality arising from membrane overloading. However, for bacterial cultivation in square-well blocks, a pH-stabilized richer growth medium may be helpful since cultures grow more slowly in blocks than in tubes.

- Prepare buffers before using a kit for the first time.

  Buffer P1: Add the provided RNase A solution to Buffer P1. Use one vial of RNase A (spin down briefly before use) per bottle of Buffer P1, to give a final concentration of 100 µg/ml. This solution is stable for 6 months when stored at 2–8°C.

  Buffer PE: Add the appropriate volume (indicated on the bottle) of ethanol to Buffer PE concentrate.

  Buffer P2: Check Buffer P2 for SDS precipitation. If necessary, redissolve the precipitate by warming. Avoid exposure of Buffer P2 to air since CO_2 can reduce its effectiveness.

- Any vacuum source may be used. Optimal vacuum is between –200 and –300 mbar for sample transfer and elution, and up to maximum vacuum for ethanol (Buffer PE) removal. See "Recommended vacuum pressures" on page 13.
- For large numbers of samples, an eight-channel pipet with a large fill-volume (≥1 ml) per channel is recommended.

Bacterial Lysis for QIAwell Ultra Plasmid Kits

A complete protocol (optional) for cultivating, harvesting, and lysing bacterial pellets in square-well blocks is given on page 17.

The following steps (1–3) apply to all QIAwell Ultra Kits:

Harvest the bacterial cells by centrifugation before beginning the procedure. Bacteria can be harvested in 2 ml microfuge tubes for 5 min at 3000 x *g* (~5500 rpm). Bacteria can also be harvested in 15 ml or 50 ml culture tubes for 5 min at 5300 x *g* (5300 x *g* corresponds to 5000 rpm in the QIAGEN Centrifuge 4 K15C).

1. **Resuspend the bacterial pellet in 0.3 ml Buffer P1.**

   Ensure that RNase A has been added to Buffer P1. The pelleted cells should be resuspended completely, leaving no cell clumps. The tubes may be vortexed briefly to accelerate resuspension.

2. **Add 0.3 ml Buffer P2, gently invert the tube 4–6 times to mix, and incubate at room temperature for 5 min.**

   Do not vortex as this will cause shearing of the bacterial genomic DNA. If necessary, continue to invert the tube until the solution becomes viscous. Do not allow the incubation to proceed for more than 5 minutes since longer incubation may result in increased amounts of open circular plasmid.

3. **Add 0.3 ml chilled Buffer P3, mix immediately but gently, and incubate on ice for 10 min.**

   To avoid localized precipitation mix the solution gently but thoroughly by inverting the tubes 5–6 times immediately after addition of Buffer P3 and occasionally during the incubation.

   Continue with the appropriate protocol from step 4:

   QIAwell 8 Ultra on page 20

   QIAwell 96 Ultra on page 25

Cell Cultivation, Harvesting, and Lysis in Square-Well Blocks (Optional)

Growing bacterial cultures in 96-well Square-Well Blocks*

Fill each well of a 2.2 ml square-well block with 1.3 ml of growth medium containing the appropriate selective agent. Inoculate each well from a single bacterial colony. Incubate for 20–24 h at 37°C with shaking at 300 rpm.

During cultivation the cultures may be protected against spill-over by covering the block with an AirPore™ Tape Sheet (see Ordering Information, pages 40–41). Alternatively, adhesive tape with 2–3 holes pierced above each well may be used. For lysis steps the wells should be covered with wide non-porous adhesive tape such as a sheet from a QIAGEN Tape Pad (see Ordering Information, pages 40–41).

Harvesting bacterial cultures in Square-Well Blocks

Harvest the bacteria by centrifuging the block for 5 min at 1500 x *g* in a centrifuge with a rotor for microtiter plates. The block should be covered with adhesive tape during centrifugation. After centrifugation, remove the tape and quickly invert the block over a waste container. Tap the inverted block firmly on a paper towel to remove any remaining medium.

Preparing bacterial lysates in Square-Well Blocks

1. **Resuspend each bacterial pellet in 0.3 ml Buffer P1. Use an 8-channel pipet with a large fill volume (≥1 ml per channel) for buffer delivery. Tape the block and mix by vortexing.**

   Ensure that RNase A has been added to Buffer P1.The cells in the wells should be resuspended completely, leaving no clumps.

   Note: Bacterial pellets can be frozen for several days without compromising plasmid yield and quality.

2. **Add 0.3 ml Buffer P2 to each well, seal the block with new tape, mix gently by inversion, and incubate at room temperature for 5 min.**

   After addition of Buffer P2, the block should be resealed and the solution mixed gently but thoroughly by inverting 10 times. Do not vortex at this step, as this may cause shearing of the bacterial genomic DNA. Do not allow the incubation to proceed for more than 5 minutes. The lysate should appear viscous, without any bacterial-cell clumps.

** For QIAwell procedures on the BioRobot 9600, cell cultivation, harvesting, and lysis should be performed in 2 ml, 96-well, flat-bottom blocks.*

3. **Add 0.3 ml Buffer P3 to each well, seal the block with new tape, and mix immediately by inversion. Incubate on ice for 10 min.**

   Ensure uniform precipitation by gently inverting the taped block 10 times.

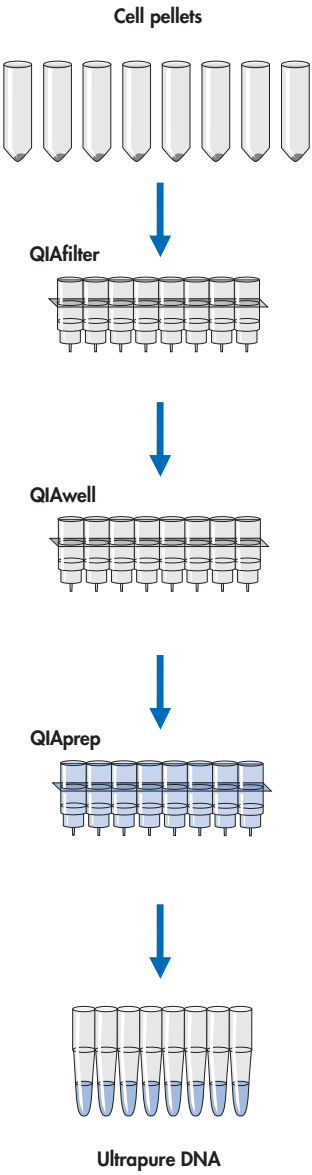
   Note: For very high-throughput processing the ice incubation can be eliminated without substantially compromising plasmid yield and quality.

Continue with the appropriate protocol from step 4:

QIAwell 8 Ultra on page 20

QIAwell 96 Ultra on page 25

QIAwell 8 Ultra Plasmid Purification Procedure



QIAwell 8 Ultra Plasmid Purification Protocol

For QIAwell 8 Ultra Plasmid Kit

Please read "Important notes before starting" on page 15 and follow steps 1–3 of the bacterial lysis protocol on page 16, or the protocol for cell cultivation, harvesting, and lysis in square-well blocks on page 17.

Clearing the lysates by filtration through QIAfilter 8 strips

4. **Prepare the QIAvac 6S vacuum manifold: place the appropriate number of (yellow) QIAfilter 8 strips in the top plate and seal any unused slots with blanks. Place the appropriate number of (clear) QIAwell 8 strips in the strip holder and place it in the base of the manifold. Assemble the manifold, making sure that the base of each QIAfilter 8 strip is aligned with the top of each QIAwell 8 strip.**

5. **Transfer the lysates from step 3 to the QIAfilter wells. Apply a vacuum between –200 and –300 mbar for 2–3 min, or until the lysates are completely transferred to the underlying QIAwell 8 strips. Turn off the vacuum source and ventilate the manifold.**

   To transfer the lysates into the QIAfilter wells, gently draw each lysate into a disposable 1 ml pipet tip by moving from the top of the liquid (where the precipitate is) toward the bottom with a circular motion. Expel the lysates gently into the wells. Unused wells may be saved by covering them with the strip caps provided or with tape.

   Note: An 8-channel pipet with a large fill-volume (≥1 ml) is recommended if square-well blocks have been used for the lysis.

6. **Discard the yellow QIAfilter 8 strips and transfer the clear QIAwell 8 strips containing the cleared lysates from the base to the top plate of the QIAvac 6S. Place the waste tray in the base and reassemble the manifold.**

Purifying plasmid DNA on QIAwell 8 strips

7. **Apply a vacuum between –200 and –300 mbar until all of the lysates have passed through the membranes (approximately 30–50 s).**

   To avoid further precipitation, the cleared lysates should be drawn through the QIAwell membranes without delay. If precipitation does occur, samples should be removed from the wells and refiltered. The solutions should pass through the membranes in 30–50 s. Transit times significantly longer than 1 min may indicate membrane clogging, while transit times of <30 s may indicate excessive vacuum.

8. **Add 1 ml Buffer QW to each well of the QIAwell 8 strips and apply a vacuum between –200 and –300 mbar for 30–50 s. Repeat this wash step once.**

   Most of the bacterial RNA and other contaminants will be removed with the first wash: the remainder will be removed with the second wash.

9. **Turn off the vacuum source, ventilate the manifold, and remove the waste tray.**

Desalting the DNA on QIAprep 8 strips

10. **Place (blue) QIAprep 8 strips into the QIAvac 6S strip holder. Add 150 µl Buffer QE to each well and incubate for 1 min.**

    Pre-wetting the QIAprep membranes will enhance the efficiency of binding.

11. **Remove the waste tray from the QIAvac base, and place the QIAvac strip holder with the QIAprep 8 strips into the manifold base, ensuring that they are aligned with the QIAwell 8 strips. Reassemble the manifold.**

12. **Add 0.7 ml Buffer QE to each well of the (clear) QIAwell 8 strips and apply a vacuum of between –200 and 300 mbar to elute the DNA from the QIAwell 8 strips into the QIAprep 8 strips. Elution should require 3–5 min.**

    Avoid excessive vacuum at this point.

13. **Ventilate the manifold. Discard the (clear) QIAwell 8 strips and transfer the QIAprep 8 strips containing the eluted DNA from the base to the top plate. Place the waste tray in the base and reassemble the manifold.**

14. **To adsorb DNA to the QIAprep membranes, apply a vacuum between –200 and –300 mbar, until all the solutions have passed through. Switch off the vacuum.**

    Switch off vacuum between addition of buffers to ensure an even pull of pressure through the wells.

15. **Wash each well of the QIAprep 8 strips with 2 x 1 ml Buffer PE, using a vacuum between –200 and –300 mbar. Continue to apply vacuum for 1 min after complete transfer of Buffer PE.**

16. **Ventilate the manifold and remove the top plate containing the QIAprep 8 strips from the manifold. Remove any traces of Buffer PE by vigorously tapping the top plate on a stack of absorbent paper.**

    Ethanol from the membranes and from the collars and nozzles of each well will be absorbed by the paper towels. Droplets adhering to the nozzles and collars should be removed with a tissue (visually inspect the walls of the wells and remove any remaining droplets by tapping).

    Optional: QIAprep 8 strips can also be removed from the QIAvac top plate and tapped on a stack of paper towels. Take care not to break the nozzles by tapping too vigorously.

17. **Place the top plate containing the QIAprep 8 strips back on the manifold, apply maximum vacuum for 1 min, ventilate, and repeat the buffer removal procedure from step 16.**

    Repeating the procedure will remove any droplets which have fallen from the walls of the wells in the first step.

    Note: Continue alternating vacuum and tapping steps until no further Buffer PE spots are observed on the absorbent paper.

18. **Place the top plate containing the QIAprep 8 strips back on the manifold and apply maximum vacuum for an additional 5 min.**

    This step will evaporate any remaining ethanol from the membranes.

    Note: Residual ethanol, if carried over, can reduce the elution efficiency and inhibit subsequent enzymatic reactions.

19. **Remove the waste tray and replace it with the blue microtube rack containing 1.2 ml collection microtubes. Reassemble the manifold, making sure that the QIAprep 8 strips and collection microtubes are properly aligned.**

20. Add 75 µl Buffer EB (10 mM Tris·Cl, pH 8.5) to the center of each well and elute DNA by applying a vacuum between –200 and –300 mbar for 30 s, then increase to –600 mbar for 30 s. Repeat with another 75 µl buffer. Turn off the vacuum, ventilate the manifold, and remove the purified DNA samples from the collection microtube rack.

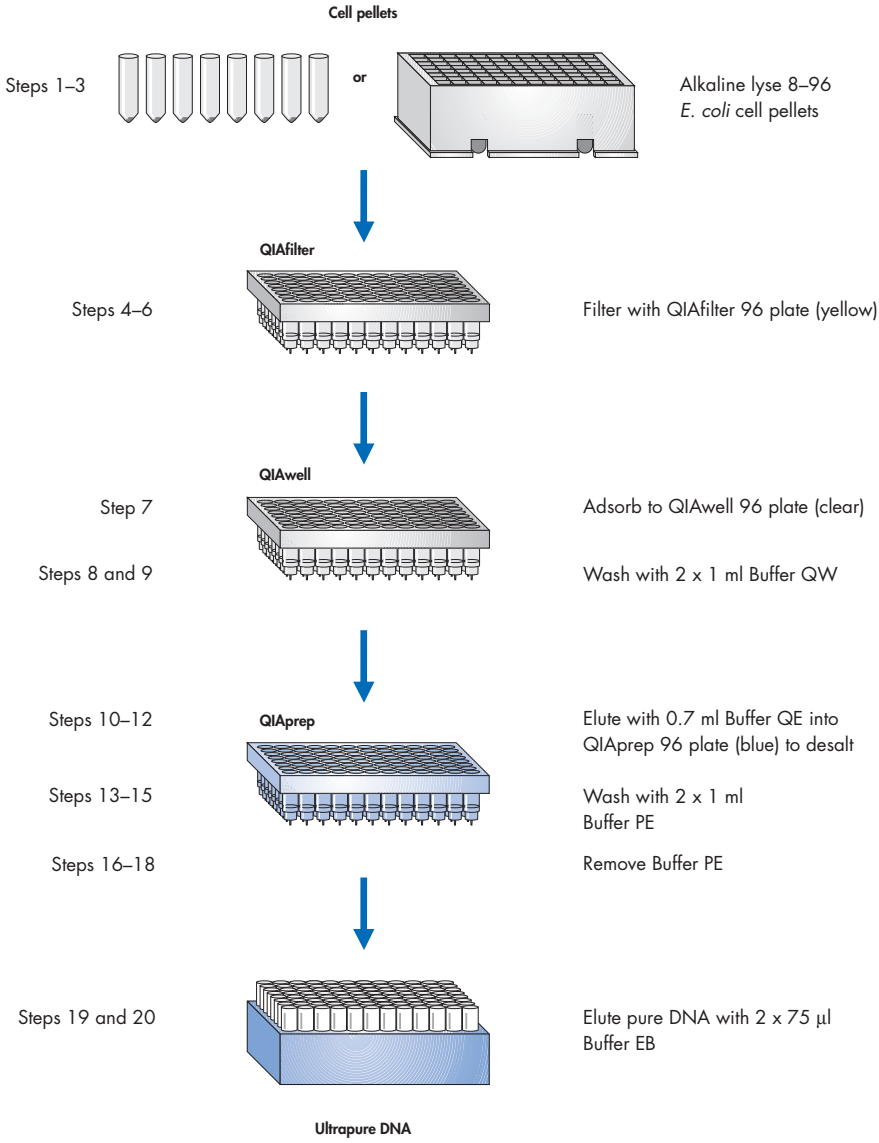
Increasing the vacuum during elution will minimize the amount of elution buffer retained on the QIAprep membrane and maximize recovery of plasmid DNA. Ensure that no spattering of sample occurs when eluting into tubes other than the QIAGEN collection microtubes supplied with the kit.

Samples can be stored in the 1.2 ml collection microtubes provided. Alternatively, the eluates may be transferred quantitatively into 1.5 ml microcentrifuge tubes by separating the 1.2 ml collection microtubes, placing them upside down in 1.5 ml microcentrifuge tubes, and microcentrifuging for approximately 3 s.

For a high-copy plasmid, DNA yields should be approximately 4–5 µg per ml of starting culture. If DNA concentrations higher than 150–200 ng/µl are required, use a smaller elution-buffer volume (see Figure 2, page 10).

If plasmid DNA is to be concentrated by drying, elute in 1 mM Tris·Cl, pH 8.5, or H_2O with a pH >7.0.

QIAwell 96 Ultra Plasmid Purification Procedure



QIAwell 96 Ultra Plasmid Purification Protocol

For QIAwell 96 Ultra Plasmid Kit

Please read "Important notes before starting" on page 15 and follow steps 1–3 of the bacterial lysis protocol on page 16, or the protocol for cell cultivation, harvesting, and lysis in square-well blocks on page 17.

Clearing the lysates by filtration on the QIAfilter 96 plate

4. **Prepare the QIAvac 96 vacuum manifold: place a (yellow) QIAfilter 96 plate into the top plate and a (clear) QIAwell 96 plate in the plate holder in the manifold base. Assemble the manifold.**

5. **Transfer the lysates from step 3 to the QIAfilter wells. Apply a vacuum between –200 and –300 mbar for 2–3 min, or until the lysates are completely transferred to the underlying QIAwell 96 plate. Turn off the vacuum source and ventilate the manifold.**

   To transfer the lysates into the QIAfilter wells, gently draw each lysate into a disposable 1 ml pipet tip by moving from the top of the liquid (where the precipitate is) toward the bottom with a circular motion. Expel the lysates gently into the wells. Unused wells may be saved by covering them with the strip caps provided or with tape.

   Note: An 8-channel pipet with a large fill-volume (≥1 ml) is recommended if square-well blocks have been used for the lysis.

6. **Discard the yellow QIAfilter 96 plate and transfer the clear QIAwell 96 plate containing the cleared lysates from the base to the top plate of the QIAvac 96. Place the waste tray in the base and reassemble the manifold.**

Purifying plasmid DNA on the QIAwell 96 plate

7. **Apply a vacuum between –200 and –300 mbar until all of the lysates have passed through the membranes (approximately 30–50 s).**

   To avoid further precipitation, the cleared lysates should be drawn through the QIAwell membranes without delay. If precipitation does occur samples should be removed from the wells and refiltered. The solutions should pass through the membranes in 30–50 s. Transit times significantly longer than 1 min may indicate membrane clogging, while transit times of <30 s may indicate excessive vacuum.

8. **Add 1 ml Buffer QW to each well of the QIAwell 96 plate and apply a vacuum between –200 and –300 mbar for 30–50 s. Repeat this wash step once.**

   Most of the bacterial RNA and other contaminants will be removed with the first wash: the remainder will be removed with the second wash.

9. **Turn off the vacuum source, ventilate the manifold, and remove the waste tray.**

Desalting the DNA on the QIAprep 96 plate

10. Place a (blue) QIAprep 96 plate into the QIAvac 96 plate holder. Add 150 µl Buffer QE to each well and incubate for 1 min.

Pre-wetting the QIAprep membranes will enhance the efficiency of binding.

11. Remove the waste tray from the QIAvac base, and place the QIAvac plate holder with the QIAprep 96 plate into the manifold base. Reassemble the manifold.

12. Add 0.7 ml Buffer QE to each well of the (clear) QIAwell 96 plate and apply a vacuum of between –200 and 300 mbar to elute the DNA from the QIAwell 96 plate into the QIAprep 96 plate. Elution should require 3–5 min.

Avoid excessive vacuum at this point.

13. Ventilate the manifold. Discard the (clear) QIAwell 96 plate and transfer the QIAprep 96 plate containing the eluted DNA from the base to the top plate. Place the waste tray in the base and reassemble the manifold.

14. To adsorb DNA to the QIAprep membranes, apply a vacuum between –200 and –300 mbar, until all the solutions have passed through. Switch off the vacuum.

Switch off vacuum between addition of buffers to ensure an even pull of pressure through the wells.

15. Wash each well of the QIAprep 96 plate with 2 x 1 ml Buffer PE, using a vacuum between –200 and –300 mbar. Continue to apply vacuum for 1 min after complete transfer of Buffer PE.

16. Ventilate the manifold and remove the top plate containing the QIAprep 96 plate from the manifold. Remove any traces of Buffer PE by vigorously tapping the top plate on a stack of absorbent paper.

Ethanol from the membranes and from the collars and nozzles of each well will be absorbed by the paper towels. Droplets adhering to the nozzles and collars should be removed with a tissue (visually inspect the walls of the wells and remove any remaining droplets by tapping).

Optional: The QIAprep 96 plate can also be removed from the QIAvac top plate and tapped on a stack of paper towels. Take care not to break the nozzles by rapping too vigorously. If a 96-well-microplate centrifuge is available, a more convenient approach to remove ethanol may be centrifugation. Tape a standard 96-well microplate to the base of the QIAprep 96 plate and centrifuge at 1300 x *g* for 1 min.

17. **Place the QIAprep 96 plate back on the manifold, apply maximum vacuum for 1 min, ventilate, and repeat the buffer removal procedure from step 16.**

    Repeating the procedure will remove any droplets which have fallen from the walls of the wells in the first step.

    Note: Continue alternating vacuum and tapping steps until no further Buffer PE spots are observed on the absorbent paper.

18. **Place the QIAprep 96 plate back on the manifold and apply maximum vacuum for an additional 5 min.**

    This step will evaporate any remaining ethanol from the membranes.

    Note: Residual ethanol, if carried over, can reduce the elution efficiency and inhibit subsequent enzymatic reactions.

19. **Remove the waste tray and replace it with the blue microtube rack containing 1.2 ml collection microtubes. Reassemble the manifold, making sure that the QIAprep 96 plate and collection microtubes are properly aligned.**

20. **Add 75 µl Buffer EB (10 mM Tris·Cl, pH 8.5) to the center of each well and elute DNA by applying a vacuum between –200 and –300 mbar for 30 s, then increase to –600 mbar for 30 s. Repeat with another 75 µl buffer. Turn off the vacuum, ventilate the manifold, and remove the purified DNA samples from the collection microtube rack.**

    Increasing the vacuum during elution will minimize the amount of elution buffer retained on the QIAprep membrane and maximize recovery of plasmid DNA. Ensure that no spattering of sample occurs when eluting into tubes other than the QIAGEN collection microtubes supplied with the kit.

    Samples can be stored in the 1.2 ml collection microtubes. Alternatively, the eluates may be transferred quantitatively into 1.5 ml microcentrifuge tubes by placing the 1.2 ml tubes upside down in 1.5 ml microcentrifuge tubes and microcentrifuging for approximately 3 s.

    The DNA samples can also be conveniently eluted into a microtiter plate using either QIAvac 96 or a microtiter plate centrifuge. To elute with the vacuum manifold, place a standard round-bottom microtiter plate on top of an empty microtube rack in the manifold. To elute by centrifugation, tape a standard 96-well microplate to the base of the QIAprep 96 plate and centrifuge for 1 minute at 1300 x *g*.

    For a high-copy plasmid, DNA yields should be approximately 4–5 µg per ml of starting culture. If DNA concentrations higher than 150–200 ng/µl are required, use a smaller elution-buffer volume (see Figure 2, page 10).

    If plasmid DNA is to be concentrated by drying, elute in 1 mM Tris·Cl, pH 8.5, or H_2O with a pH >7.0.

Troubleshooting Guide

This troubleshooting guide, as well as the information provided in the appendices of this handbook may be helpful in solving any problems which may arise. The scientists at QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocol(s) in this handbook or molecular biology applications (see inside front cover for contact information).

	Comments and suggestions

Low or no DNA yield

General

Low yields and impure DNA may be caused by a number of different factors. Running a small analytical gel of fractions saved from each step is the best way to identify where losses may be occurring (Appendix C, page 38). Approximately 10% of the cleared lysate and the eluates, and the entire flow-through can be precipitated by adding 0.7 volumes isopropanol and centrifuging at maximum speed (10,000 x *g* or 13,000 rpm) for 30 min. 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.

Sequence related

Problems related to quality in fluorescent sequencing may need specialized troubleshooting with respect to the specific sequencing system used. *The QIAGEN Guide to Template Purification and DNA Sequencing* (2nd Edition) contains useful information on sequencing optimization and troubleshooting, and is available free on request.

No DNA in the cleared lysate before loading

a)	Plasmid did not propagate	Please read section "Growth of bacterial cultures" (page 35) and check that the conditions for optimal growth were met.
b)	Lysate was prepared incorrectly	Check age and storage conditions of buffers. If necessary, use fresh Buffers P1, P2, and P3.
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 fraction

a)	QIAwell membrane overloaded	Do not use rich media (except when growing cultures in square-well blocks). Do not exceed the recommended culture volumes. It may even be necessary to adjust LB culture volume if the plasmid and host strain show exceptionally high copy number or growth rates (see "Culture media", page 36).
b)	RNase A digestion insufficient or omitted	Check culture volume and reduce if necessary. If Buffer P1 is more than 3 months old, add more RNase A.
c)	SDS (or other ionic detergent) in lysate	Make sure that Buffer P3 is chilled before use, and the lysate is incubated on ice before centrifugation. Load cleared lysate onto the column promptly after centrifugation. If lysate is too viscous for effective mixing of Buffer P3, reduce culture volume or increase volumes of Buffers P1, P2, and P3.
d)	Lysis buffers incompletely mixed	Ensure complete mixing of all buffers.

DNA is found in the wash fraction

Wash buffer was incorrect	Ensure that Buffer QW and Buffer PE were applied according to the protocol.

No DNA in eluate

Elution buffer was incorrect	Use Buffer QE for elution from QIAwell modules and Buffer EB for elution from QIAprep modules. If water is used for elution from QIAprep modules, ensure that it has pH >7.0, as elution efficiency is dependent on the pH.

Contaminated DNA/poor-quality DNA

RNA in the eluate

a)	RNase A digestion is insufficient	Check culture volume, and reduce if necessary. If Buffer P1 is more than 3 months old, add more RNase A. Increase wash-buffer volume.

Genomic DNA in the eluate

a)	Mixing too vigorous	The lysate must be handled gently after addition of Buffer P2, to prevent shearing of DNA. Reduce culture volume if lysate is too viscous for gently mixing.
b)	Lysis time too long	Ensure that the lysis step does not exceed 5 min.
c)	Cell lysis during culture	Do not grow cultures for more than 12–16 hours (except when cultivating in square-well blocks).

DNA is nicked/sheared/degraded

a)	Endonuclease-containing host strain	Refer to "Host strains" (page 35), and consider changing *E. coli* strain.
b)	Nuclease contamination	Check buffers for nuclease contamination and replace if necessary. Use autoclaved glass and plastic, and wear gloves.
c)	DNA poorly buffered	Resuspend DNA in 10 mM Tris·Cl, pH 8.5 (Buffer EB in QIAwell Ultra Plasmid Kits) to maintain stable pH during storage.
d)	Shearing during resuspension	Resuspend DNA gently, without vigorous vortexing or pipetting.

DNA does not perform well

a)	DNA was nicked	Check percentage of nicked DNA in lysate on an agarose gel. If significant nicked DNA is present, reduce culture volume or use an alternative host strain.
b)	Salt concentration too high	Check that Buffer PE washing steps were carried out correctly.
c)	Residual proteins in DNA	Check culture volume, medium, and host-strain growth. Increase volume of wash buffer.

d)	Ethanol in DNA	Ethanol may have been carried over from Buffer PE washes into the eluate. Ensure that the steps for removal of ethanol droplets are carried out carefully as recommended. If ethanol appears to be a problem the DNA can be precipitated or lyophilized without lowering DNA quality. Complete removal of ethanol can be checked by comparing the weight of the QIAprep modules before use (step 10) and after ethanol removal steps.

DNA contains extra bands on an analytical gel

a)	Possible deletion mutants	Some sequences are poorly maintained in plasmids. Check for deletions by restriction analysis.
b)	Plasmid has formed denatured supercoils	These species run faster than closed circular DNA on a gel and are resistant to cleavage. Incubate cells for no longer than 5 min in Buffer P2.
c)	Plasmid multimers are present	Digest DNA with a restriction enzyme with a unique site in the plasmid. Monomers and multimers will form one band on an agarose gel (see Appendix C, page 38).

Blocked QIAwell module positions

a)	Lysate was turbid	Ensure that the supernatant is clear before it is loaded onto the QIAwell module. Chill Buffer P3 and incubate the lysate on ice before centrifugation. Repeat centrifugation step to ensure all debris and SDS are pelleted.
b)	Salt precipitation in the well	Allow lysates to pass through the QIAwell membrane completely before applying the wash buffer.

Appendix A: QIAwell and QIAGEN Anion-Exchange Technology

QIAwell unites patented QIAGEN Anion-Exchange Resin with Empore® membrane technology from 3M. QIAGEN Resin particles are enmeshed in an inert PTFE matrix, combining the high capacity and selectivity of QIAGEN purification with the handling advantages of a membrane.

QIAGEN Resin is a unique anion-exchanger with a hydrophilic surface modification that selectively separates nucleic acids from other substances, such as proteins, carbohydrates, metabolites, or dyes. The macroporous anion-exchanger, with a particle size of approximately 100 µm, has a hydrophilic surface-coating containing DEAE groups, creating an extremely high surface-charge density. The large pore size, together with the high density of anion-exchange groups, provide the extraordinarily broad separation range (Figure 5) that makes QIAGEN Resin unique.

The separation range of QIAGEN Resin extends from 0.1 M to 1.6 M salt. Conventional anion-exchange resins, based on cellulose, dextran or agarose, have separation ranges up to only 0.4 M salt. Binding and elution of all substances is thus limited to a narrow range of salt concentrations. As the elution peaks of proteins, RNA, and DNA overlap extensively with one another, a satisfactory separation cannot be achieved. The separation and purification qualities of QIAGEN Resin, as well as its ease of use, are thus far superior to conventional anion-exchange resins.

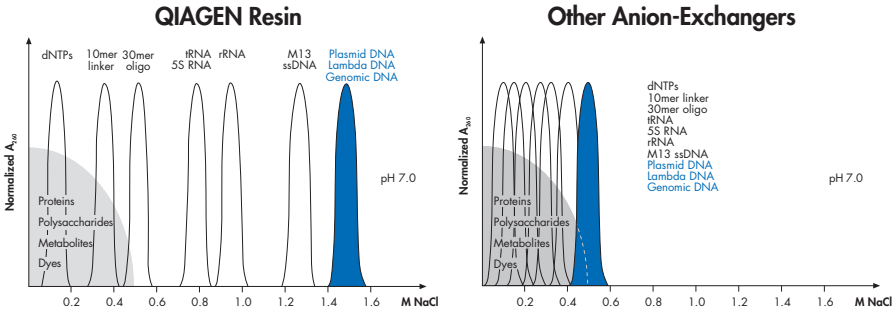


Figure 5. *Separation of nucleic acids at neutral pH on anion-exhange resins.*

DNA purity and biological activity

Nucleic acids prepared using QIAGEN anion-exchange chromatography are of comparable purity to nucleic acids prepared by two rounds of purification on CsCl gradients. No protein contamination is detectable with even the most sensitive detection methods. DNA prepared using QIAGEN anion-exchange chromatography has been tested with all common restriction endonucleases, polymerases (including *Taq* DNA polymerase), DNA ligases, phosphatases, and kinases. Results were comparable to those achieved using CsCl-prepared DNA. Subsequent procedures such as transfection, transformation, sequencing, cloning, and in vitro transcription and translation proceed with optimal efficiency.

Capacity and recovery

The QIAwell System components are designed with a maximum binding capacity of 20 µg of double-stranded DNA per well (as determined with pUC18 DNA). Actual DNA yield may vary depending on the various factors described in Appendix B (page 35).

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

Buffers

The binding, washing, and elution conditions for the QIAwell membrane are strongly influenced by pH. QIAwell modules will not function in the presence of anionic detergents such as SDS, or at a pH less than 4.0. Figure 6 shows the influence of pH on the salt concentration required for elution of various types of nucleic acids. Deviations from the appropriate pH values of the buffers at a given salt concentration may result in losses of the desired nucleic acid.

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

All relevant buffers and solutions are included in the full range of QIAwell Kits to maximize yield of nucleic acid.

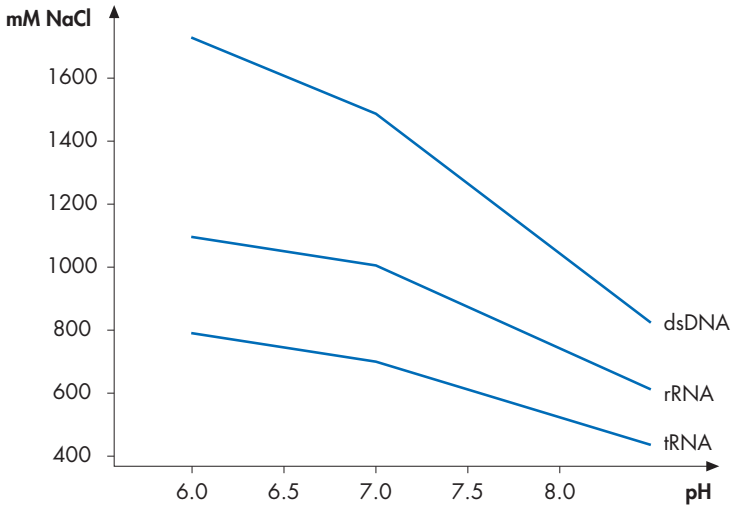


Figure 6. *Elution points of different nucleic acids from QIAGEN Resin as a function of pH and salt concentration.*

Appendix B: The QIAwell Plasmid Purification Procedure

Growth of bacterial cultures

Plasmids are usually prepared from bacterial cultures grown in the presence of a selective agent such as an antibiotic (1,2). The yield and quality of prepared 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, depending on the type of origin of replication they contain (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 (Table 5). 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 are generally present in lower copy numbers, while very large plasmids are often maintained at very low copy numbers per cell.

Table 5. Origins of replication and copy numbers of various plasmids

Plasmid	Replicon	Copy number
pUC vectors	pMB1*	500–700
pBluescript® vectors	ColE1	300–500
pGEM® vectors	pMB1*	300–400
pTZ	pMB1*	>1000
pBR322 and its derivatives	pMB1*	15–20
pACYC and its derivatives	p15A	10–12
pSC101 and its derivatives	pSC101	~5

** 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 can have a substantical influence on the quality of the purified DNA. Host strains such as DH1, DH5α™, and C600 yield high-quality DNA with the QIAwell system. 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, TG2 and the JM100 series, contain large amounts of carbohydrate which are released during lysis and can inhibit enzyme activities (1,2) 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 than that prepared from strains such as XL1-Blue, DH1, DH5α, and C600. The methylation and growth characteristics of the strain can also affect plasmid isolation. 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 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. A single colony should be inoculated into 1–5 ml of medium containing the appropriate selective agent, and grown for 12–16 hours (late logarithmic phase). Growth for longer periods is not recommended since cells begin to lyse and plasmid yield and quality 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 evenly during cell division. Daughter cells which 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 considered when determining the working concentration. 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 being depleted. This phenomenon is clearly demonstrated on ampicillin plates, where satellite colonies are seen as the ampicillin becomes hydrolyzed in the vicinity of a growing colony. Ampicillin is also sensitive to temperature, and stock solutions should be stored frozen in single-use aliquots.

Culture media

The QIAwell System protocols are optimized for use with cultures grown in standard Luria Bertani (LB) medium*, to a cell density of approximately 1.5–2 x 10^9 cells per ml (approximately 1.5–2.0 A_{600} units/ml). It is not necessary to use super rich growth media such as TB (terrific broth) or 2x YT for most commonly used high-copy plasmids. These rich media will lead to extraordinarily high cell densities; cultures grown in TB may yield 2–5 times the number of cells as those grown in LB. If these media are used, the culture volumes must be reduced according to the cell density measured to match the capacity of the QIAwell System, and growth time should be reduced to 8–10 hours. Furthermore, the excessive viscosity of the lysate would require vigorous mixing, which can cause shearing of bacterial genomic DNA and contamination of the plasmid DNA. In addition, differences in flow rates through the QIAfilter and QIAwell modules may result from overloading. Care must also be taken if strains are used which grow to very high cell densities or which grow unusually fast. It is best to calculate cell number in the culture, and adjust the volumes accordingly. Alternatively, a titration of different culture volumes per well (i.e., 0.5 to 5 ml) will identify optimal culture volumes for both yield and plasmid quality. For bacterial cultivation in square-well blocks, a pH-stabilized richer growth medium may be helpful since bacterial cultures grow more slowly in blocks than in tubes.

** LB medium has the following composition per liter: 10 g tryptone, 5 g yeast extract, and 10 g NaCl.*

Preparation of cleared lysates

After harvesting and resuspension in Buffer P1, the bacterial cells are lysed in NaOH/SDS, (Buffer P2) in the presence of RNase A (3, 4). SDS solubilizes the phospholipid and protein components of the cell membrane, leading to lysis and release of the cell contents. NaOH denatures the chromosomal and plasmid DNAs, as well as proteins. The optimized lysis time allows maximum release of plasmid DNA without release of chromosomal DNA, while minimizing the exposure of the plasmid to denaturing conditions. Long exposure to alkaline conditions may cause the plasmid to become irreversibly denatured. This denatured form of the plasmid runs faster on agarose gels and is resistant to restriction enzyme digestion (see Figure 7, page 38).

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

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 the insoluble complexes containing salt, detergent, and protein. Plasmid DNA remains in the clear supernatant. Vigorous treatment during the lysis procedure will shear the bacterial chromosome, leaving free chromosomal DNA fragments in the supernatant. Since chromosomal fragments are chemically indistinguishable from plasmid DNA under the conditions used, the two species will not be separated on QIAGEN Resin and will elute under the same salt conditions. Mixing during the lysis procedure should therefore be carried out only by gentle inversion.

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

Determination of yield

Plasmid yield will depend on the copy number of the plasmid and the culture volume. Yields are best determined by measuring DNA concentration on a fluorimeter or spectrophotometer, followed by visual examination against a known standard DNA on an agarose gel. Readings on a spectrophotometer are not always accurate, particularly if a single wavelength measurement is taken rather than a scan.

** KDS: Potassium dodecyl sulfate*

Appendix C: Agarose Gel Analysis of Plasmid DNA

A 1% analytical agarose gel of fractions at each stage of a QIAwell purification procedure is shown in Figure 7. In addition, the gel shows some general purification artifacts, which may be visible under certain plasmid-purification conditions. The entire procedure can be easily monitored by saving equal proportions of each fraction and precipitating the nucleic acids with isopropanol before loading them onto the gel.

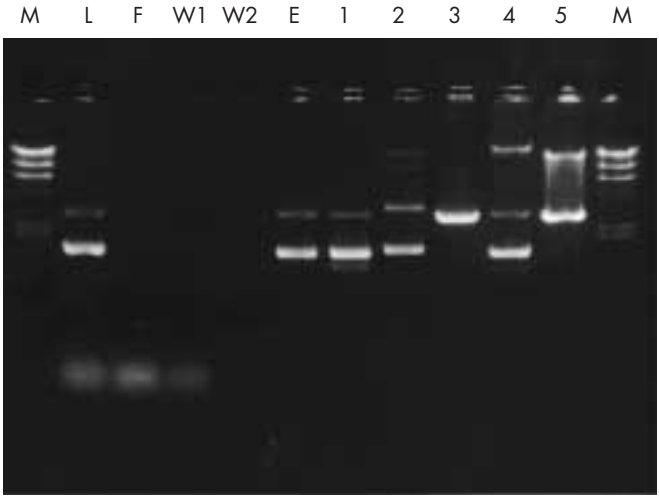


Figure 7. *Analytical agarose gel.*

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

F: Flow-through fraction containing only degraded RNA. Plasmid DNA is bound to the QIAGEN Resin.

W1: First wash fraction, in which the remaining traces of RNA are removed without affecting the binding of the DNA.

W2: Second wash fraction, which ensures that the resin is completely cleared of RNA and other contaminants, leaving only pure plasmid DNA on the column.

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

M: Lambda DNA digested with *Hin*dIII.

Lanes 1–5 demonstrate some atypical results that may be observed in some preparations, depending on plasmid type and host strain.

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.

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 one defined band with the size of the linearized plasmid monomer (see lane 3).

3: Linearized form of plasmid pTZ19 after restriction digestion with *Eco*RI.

4: Sample contaminated with bacterial chromosomal DNA, which may be observed if the lysate is treated too vigorously, e.g., vortexing during incubation steps with Buffer P2 or P3. Genomic DNA contamination can easily be identified by digestion of the sample with *Eco*RI. A smear is observed, in contrast to the linear band seen after digestion of multimeric plasmid forms.

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

References

1. *Sambrook, J., and Russell, D., eds. (2001) Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press.*
2. *Ausubel F.M.*. et al.*, eds. (1999) Current protocols in molecular biology. Wiley Interscience, New York.*
3. *Birnboim, H.C. and Doly, J. (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucl. Acids Res.* ***7****, 1513-1522.*
4. *Birnboim, H.C. (1983) A rapid alkaline extraction procedure for the isolation of plasmid DNA. Methods Enzymol.* ***100****, 243-255.*

Ordering Information

Product	Contents	Cat. No.
QIAwell 8 Ultra Plasmid Kits		
QIAwell 8 Ultra Plasmid Kit (10)*	For 10 x 8 ultrapure plasmid minipreps, 10 each: QIAfilter 8, QIAwell 8, and QIAprep 8 Strips; Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	16152
QIAwell 96 Ultra Plasmid Kits		
QIAwell 96 Ultra Plasmid Kit (4)‡	For 4 x 96 ultrapure plasmid minipreps, 4 each: QIAfilter 96, QIAwell 96, and QIAprep 96 Plates; Reagents, Buffers, Collection Microtubes (1.2 ml), Caps	16191
QIAwell 96 Ultra BioRobot Kit (4)†	For 4 x 96 ultrapure plasmid minipreps, 4 each: QIAfilter 96, QIAwell 96, and QIAprep 96 Plates; Flat-Bottom Blocks and Lids, Reagents, Buffers, Collection Microtubes (1.2 ml), Caps, 96-Well Microplates and Lids, Tape Pads	960141
QIAvac Manifolds		
QIAvac 6S	Vacuum manifold for processing of 1–6 QIAGEN 8-well strips: includes QIAvac 6S Top Plate with flip-up lid, Base, Waste Tray, Microtube Rack, Blanks, and Strip Holder	19503
QIAvac 96	Vacuum manifold for processing of QIAGEN 96-well plates: includes QIAvac 96 Top Plate, Base, Waste Tray, and Plate Holder	19504
Centrifuges		
Centrifuge 4-15C	Universal laboratory centrifuge with brushless motor	Inquire
Centrifuge 4K15C	Universal refrigerated laboratory centrifuge with brushless motor	Inquire

* *Requires use of QIAvac 6S.*
† *Special kit format for automated minipreps on the BioRobot 9600.*
‡ *Requires use of QIAvac 96.*

Ordering Information

Product	Contents	Cat. No.
Accessories		
Vacuum Regulator	For use with QIAvac manifolds	19530
Collection Microtubes (racked)	Nonsterile polypropylene tubes (1.2 ml), 960 in racks of 96	19560
Collection Microtube Caps	Nonsterile polypropylene caps for Collection Microtubes (1.2 ml), 960 in strips of 8, loose in bag	19566
Square-Well Blocks (24)	96-well blocks with 2.2 ml wells, 24 blocks per case	19573
Flat-Bottom Blocks (8)	96-well blocks with 2 ml wells, 8 blocks per case	19578
Flat-Bottom Blocks (24)	96-well blocks with 2 ml wells, 24 blocks per case	19579
96-Well Microplates RB (24)	96-well microplates with round-bottom wells plus lids, 24 per case, for use with QIAvac manifolds and the BioRobot 9600	19581
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack	19570
AirPore Tape Sheets (50)	Microporous tape sheets for covering 96-well blocks during bacterial cultivation: 50 sheets per pack	19571
Buffer QF	1000 ml elution buffer	19056
Buffer QW	1000 ml wash buffer	19067

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