September 2003

QIAquick® Multiwell PCR Purification Handbook

For rapid purification of multiple PCR products

QIAGEN

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Contents

Kit Contents

QIAquick® 8 PCR Purification Kit	**(50)**
Catalog no.	**28144**
Number of preps	**50**
QIAquick 8 Strips	50
Buffer PB*	500 ml
Buffer PE (concentrate)†	2 x 100 ml
Buffer EB	2 x 55 ml
Collection Microtubes (1.2 ml)	55 x 8
Caps for Collection Microtubes	55 x 8
Caps for Strips	55 x 8
Handbook	1

QIAquick 96 PCR Purification Kit‡	**(4)**	**(24)**
Catalog no.	**28181**	**28183**
Number of preps	**4**	**24**
QIAquick 96 Plates	4	24
Buffer PM*	500 ml	3 x 500 ml
Buffer PE (concentrate)†	2 x 100 ml	2 x 200 ml, 1 x100 ml
Buffer EB	55 ml	1 x 250 ml, 1 x 55 ml
RNase-free water	50 ml	250 ml
Collection Microtubes, (1.2 ml) (in microtube racks)	4 x 96	26 x 96
Caps for Collection Microtubes	55 x 8	6 x (55 x 8)
Handbook	1	1

* Contains a guanidine salt. Not compatible with disinfecting reagents containing bleach. See page 6 for safety information.

† Buffer PE is supplied as a concentrate. Before using for the first time, add ethanol (96–100%) as indicated on the bottle to obtain a working solution.

‡ Larger kit sizes also available; please inquire.

Storage

QIAquick 8 and 96 PCR Purification Kits should be stored dry at room temperature (15–25°C). Under these conditions they can be stored for up to 12 months without reduction in performance and quality. For longer storage, these kits can also be kept at 2–8°C, but in this case the buffers should be redissolved before use. Make sure that all buffers are at room temperature when used.

Product Use Limitations

QIAquick 8 and 96 PCR Purification 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.

Quality Control

As part of the stringent QIAGEN quality assurance program, the performance of QIAquick 8 and 96 PCR Purification Kits is monitored routinely and on a lot-to-lot basis. Kits are tested by isolation of PCR products of various sizes from amplification reactions. The quality of the isolated DNA is checked by several assays commonly used for nucleic acids. The DNA binding capacity of QIAquick strips and plates is tested by determining the recovery from a specific amount of loaded DNA.

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.

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

In case 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 the components of the QIAquick 8 and 96 PCR Purification Kits.

Buffer PB

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

Buffer PM

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

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; S13: Keep away from food, drink, and animal feedingstuffs; S23: Do not breathe vapor; S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36: Wear suitable protective clothing; 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.

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 QIAquick 8 and 96 PCR Purification 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 page).

Specifications

Binding capacity of membrane (ds DNA):	10 µg
Separation specifications:	100 bp – 10 kb products from 40mer primers
Recovery (100 bp – 10 kb):	80–95% (depending on elution volume)
Minimum eluate volume:	40 µl

Introduction

The QIAquick Multiwell PCR Purification system is designed to provide manual or fully automated medium- to high-throughput PCR purification. The QIAquick Multiwell PCR Purification system consists of two kit formats:

- **QIAquick 8 PCR Purification Kits** utilize 8-well strips for purification of 8–48 PCR reactions. The rapid procedure enables purification of 48 reactions in 15 minutes on a QIAvac 6S vacuum manifold.
- **QIAquick 96 PCR Purification Kits** utilize 96-well plates for high-throughput purification of PCR products. Using QIAvac 96, as many as 96 amplification reactions can be processed in parallel in 25 minutes.

QIAquick 96 PCR Purification Kits can also be used for high-throughput purification of DNA fragments from standard or low-melt agarose gels in TAE or TBE. For this application, a protocol and an additional solubilization and binding buffer are available. Please contact our Technical Service Department for further information (see back cover).

DNA cleanup using QIAquick chemistry can be automated using QIAGEN BioRobot® Systems (see Appendix, page 25).

QIAquick Multiwell PCR Purification Kits provide high yields of pure nucleic acids, ideally suited for direct use in many applications such as:

- Sequencing
- Microarray analysis

This handbook contains technical information about the QIAquick Multiwell PCR Purification system to help users gain maximum benefit from the products. The handbook explains the technology underlying QIAquick Multiwell PCR purification and outlines the major steps of the procedures. Detailed protocols are included, as well as a Troubleshooting Guide to help with any difficulties. For further information, please contact our Technical Service Department.

Please spend some time reading this handbook and familiarizing yourself with the QIAquick Multiwell PCR Purification system.

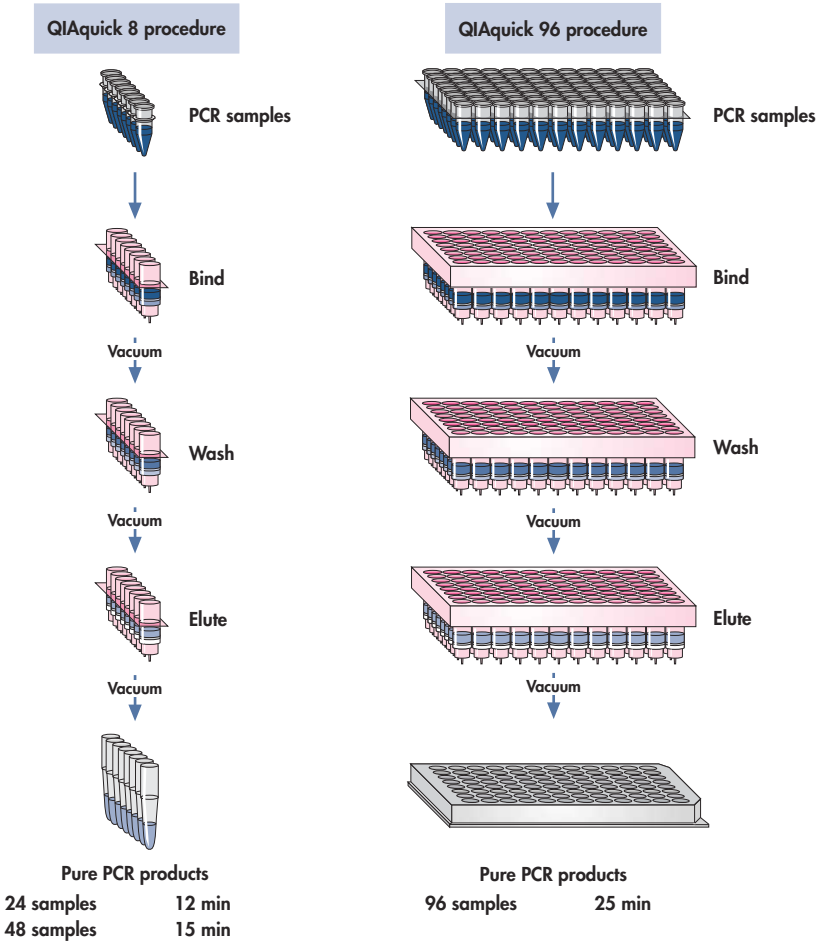
QIAquick purification

With the microarray market in mind, QIAGEN has changed the binding buffer of the QIAquick 96 PCR Purification Kits. By replacing Buffer PB with Buffer PM, the total volume of PCR sample that can be processed per well has been increased to 250 µl, as only three volumes of the buffer must be added to the sample. This larger volume enables pooling of PCR samples and, in combination with the 10 µg binding capacity, facilitates higher end-concentrations of DNA.

Furthermore, QIAquick 96 PCR Purification Kits now contain RNase-free water for elution, in addition to Buffer EB (Tris·Cl). The inclusion of RNase-free water was in response to customers who require salt-free, high-purity DNA for spotting.

Principle and procedure

The QIAquick Multiwell PCR Purification system combines the convenience of multiwell technology with the selective binding properties of a uniquely designed silica-gel membrane. The binding buffer provided with the kits is optimized for the efficient recovery of single- and double-stranded DNA fragments from 100 bp to 10 kb directly from amplification reactions, and for the quantitative (99.5%) removal of primers under 40 bases and unincorporated nucleotides. The sample is drawn through the silica-gel membrane using a vacuum, and DNA adsorbs to the silica-membrane in the presence of high salt while contaminants pass through (see flowchart, page 10). Impurities are efficiently washed away, and the pure DNA fragment is eluted with Tris buffer (Buffer EB) or water (see page 12). Since elution is performed with low-salt buffer or water, the DNA is immediately ready for use — no need to precipitate. Since it is not necessary to remove mineral oil from the amplification reaction before QIAquick purification, a multichannel pipet can be used for all pipetting steps. The simple and fast procedure makes QIAquick kits ideally suited for cleaning up DNA from many applications, such as PCR, cDNA synthesis reactions, or ligation reactions.


QIAquick 8 procedure
PCR samples
Bind
Vacuum
Wash
Vacuum
Elute
Vacuum
Pure PCR products
24 samples 12 min
48 samples 15 min
QIAquick 96 procedure
PCR samples
Bind
Vacuum
Wash
Vacuum
Elute
Vacuum
Pure PCR products
96 samples 25 min

Adsorption to QIAquick membrane — salt and pH dependence

The QIAquick silica-gel membrane is uniquely designed to isolate DNA from aqueous solutions. Up to 10 µg DNA can bind to each QIAquick membrane giving recoveries within the specifications (80–90% recovery). The binding buffer in QIAquick 8 and 96 PCR Purification Kits provides the correct salt concentration and pH for adsorption of DNA to the QIAquick membrane. The adsorption of nucleic acids to silica-gel surfaces occurs only in the presence of a high concentration of chaotropic salt (1, 2).

Adsorption of DNA to silica also depends on pH. Binding efficiency during the adsorption step is typically 95% if the pH is ≤7.5, and is reduced drastically at higher pH (Figure 1). If the loading mixture pH is >7.5, the optimal DNA-binding pH can be obtained by adding a small volume of 3 M sodium acetate, pH 5.0.

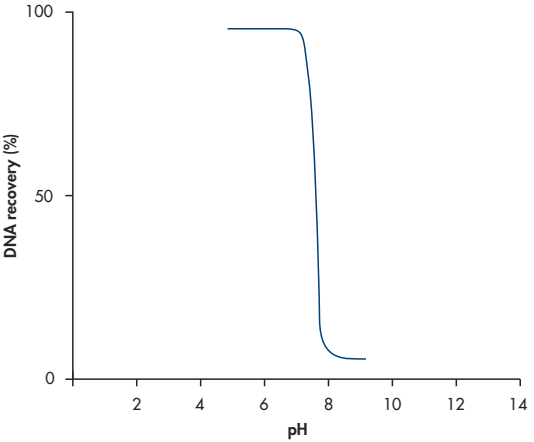


Figure 1 pH dependence of DNA adsorption to silica. 1 µg of a 2.9 kb fragment was adsorbed at different pH values and eluted with 10 mM Tris·Cl, pH 8.5. The graph shows the percentage of DNA recovery, reflecting the relative adsorption efficiency, versus the pH of adsorption.

1. Vogelstein, B. and Gillespie, D. (1979) Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA ***76***, 615–619.
2. Hamaguchi, K. and Geiduschek, E.P. (1962) The effect of electrolytes on the stability of deoxyribonucleate helix. J. Am. Chem. Soc. ***84***, 1329–1337.

Washing

During the DNA adsorption step, unwanted primers and impurities, such as salts, enzymes, unincorporated nucleotides, agarose, dyes, oils, and detergents (e.g., DMSO, Tween® 20) flow through the silica-gel membrane without binding. Salts are quantitatively washed away by the ethanol-containing Buffer PE. Any residual Buffer PE, which may interfere with subsequent enzymatic reactions, is removed by applying maximum vacuum for 5–10 minutes. Maximum vacuum generates maximum airflow through the membrane to evaporate residual ethanol.

Elution in low-salt solutions

Elution efficiency is strongly dependent on the salt concentration and pH of the elution buffer. Contrary to adsorption, elution is most efficient under basic conditions and low-salt concentrations. DNA is typically eluted with 60–80 µl low-salt solution to yield 40–60 µl of eluate.

Elution Buffer EB (10 mM Tris·Cl, pH 8.5) is included in QIAquick 8 and QIAquick 96 PCR Purification Kits to ensure optimal elution of DNA fragments from the QIAquick membrane.

Elution with TE* (10 mM Tris·Cl, 1 mM EDTA, pH 8.0) is possible, but not recommended because EDTA may inhibit subsequent enzymatic reactions.

Alternatively, DNA can be eluted in water. If using water for elution, we recommend using the RNase-free water provided in the QIAquick 96 PCR Purification Kit. If water from other sources is used, ensure that the pH is between pH 7.0 and pH 8.5.

DNA yield and concentration

DNA yield depends on the following three factors: the volume of elution buffer, the way the buffer is applied to the membrane, and the incubation time of the buffer on the membrane. 100 µl of elution buffer is sufficient to completely cover the QIAquick membrane, ensuring maximum yield, even when not applied directly to the center of the membrane. Elution with 60–80 µl requires the buffer to be added directly to the center of the membrane. The average eluate volume is 60 µl from 80 µl buffer volume, and 40 µl from 60 µl buffer volume. Smaller elution buffer volumes give higher DNA concentrations in the eluate. For example, elution with 60 µl buffer will give a 1.5-times higher DNA concentration than elution with 80 µl buffer.

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

Equipment and Reagents to Be Supplied by User

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

For all protocols

- Ethanol (96–100%)
- Disposable gloves
- Vacuum manifold (e.g., QIAvac 6S, cat. no. 19503 or QIAvac 96, cat. no. 19504)
- Vacuum source
- Optional: A vacuum regulator (e.g., cat. no. 19530) for easy monitoring of vacuum pressure and easy release of vacuum
- Optional: A reservoir or multichannel pipet facilitates liquid handling at many steps of the QIAquick 8 PCR Purification procedure. For example, the Matrix Impact® cordless multichannel pipet or the Matrix Multi-8 Electrapette® have an expandable tip-spacing system, allowing the user to transfer liquid directly from tubes to multiwell microplates.

  These can be purchased from the following distributors:

  In the USA, Germany, the UK, and Switzerland: Matrix Technologies Corporation, www.matrixtechcorp.com

  For distributors in other countries, contact Matrix Technologies Corporation, USA.
- Optional: A 96-well microplate for collection of eluted DNA

Important Notes

Preparation of buffers

Buffer PE

Add ethanol (96–100%) to the bottle containing Buffer PE before use (see bottle label for volume). Tick the check box on the bottle to indicate that ethanol has been added.

Buffer PE should be stored tightly capped to prevent evaporation of ethanol.

Handling guidelines for QIAvac 6S and QIAvac 96

QIAvac 6S and QIAvac 96 facilitate DNA cleanup by providing a convenient modular vacuum manifold for use with the QIAquick Multiwell PCR Purification System.

The following guidelines should be followed when working with QIAvac 6S or QIAvac 96.

- QIAvac 6S and QIAvac 96 operate with house vacuum, a vacuum pump, or a water aspirator. Optimum vacuum for QIAquick strips on QIAvac 6S is –200 to –600 mbar for sample transfer and up to the maximum attainable vacuum for ethanol (Buffer PE) removal. Optimum vacuum for a QIAquick 96 Plate on QIAvac 96 is –100 to –600 mbar for sample transfer and up to the maximum attainable vacuum for ethanol (Buffer PE) removal.
- Always place QIAvac 6S or QIAvac 96 vacuum manifolds on a secure bench top or work area. If dropped, the manifolds may crack.
- For safety reasons, do not use 96-well plates that are damaged.
- Always store QIAvac 6S or 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 then need to be replaced. Do not use abrasives or solvents.
- The components of QIAvac 6S and QIAvac 96 manifolds are not resistant to ethanol, methanol, or other concentrated alcohols. Do not expose the clear acrylic components of the QIAvac 6S or QIAvac 96 manifold (top plate, blanks, and strip or plate holder) to alcohol-containing reagents for long periods. Extended exposure of acrylic to ethanol will cause surface cracking. Ensure that no Buffer PE, which contains ethanol, remains in the vacuum manifold after use. Neither acrylic nor Delrin® (QIAvac 6S or QIAvac 96 base) are resistant to phenol or chloroform. If these solvents are spilled on the unit, rinse thoroughly with water.
- To ensure consistent performance, do not apply silicone or vacuum grease to any part of QIAvac 6S or QIAvac 96. The spring lock on the QIAvac 6S 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.
- Use the blanks provided to close unused slots of QIAvac 6S. Blanks are stored in the underside of the QIAvac base plate when not in use (Figure 2). Do not store blanks in slots of the QIAvac 6S top plate as this can cause deformation of the gasket.
- Always use caution and wear safety glasses when working near a vacuum manifold under pressure.
- If you have any questions regarding the performance or maintenance of your QIAvac manifold, please contact QIAGEN Technical Services or your local distributor.

QIAvac 6S and QIAvac 96 Vacuum Manifolds

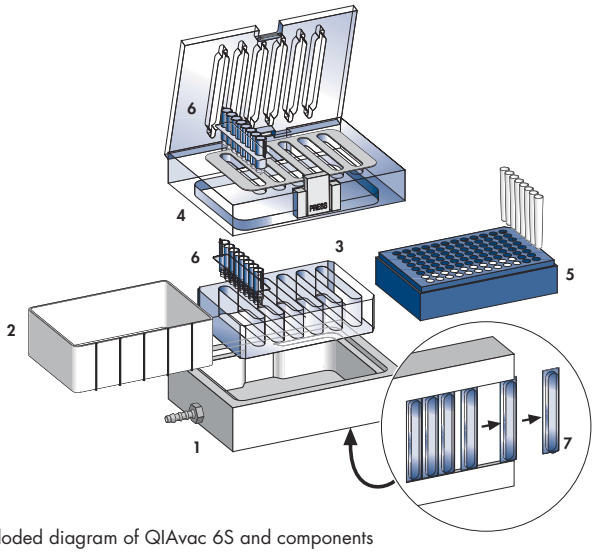


Figure 2 Exploded diagram of QIAvac 6S and components

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

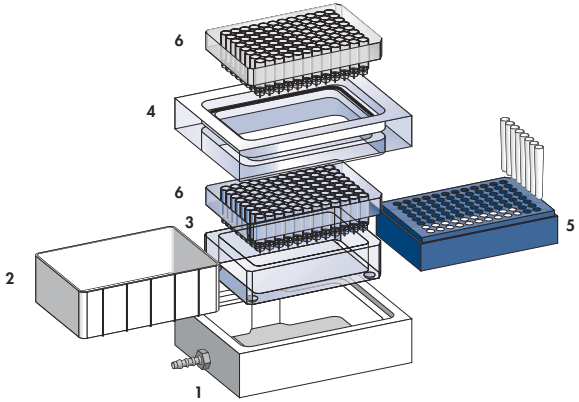


Figure 3 Exploded diagram of QIAvac 96 and components

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. Microtube rack
6. 96-well plate*

* Not included with QIAvac. Included in the appropriate QIAquick Multiwell PCR Purification Kit.

Recommended vacuum pressures

- Optimum vacuum for QIAquick strips on QIAvac 6S is –200 to –600 mbar for sample transfer and up to maximum vacuum for ethanol (Buffer PE) removal. Optimum vacuum for a QIAquick 96 plate on QIAvac 96 is –100 to –600 mbar for sample transfer 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 1.
- The vacuum pressure is the pressure differential between the inside of the manifold and the atmosphere (standard atmospheric pressure is 1013 mbar or 760 mm Hg) and can be measured by using a vacuum regulator (see ordering information, page 28). Vacuum recommendations are given in negative units (Table 1) to indicate the required reduction in pressure with respect to the atmosphere. Table 2 provides pressure conversions to other units.
- Use of excessive vacuum can cause insufficient binding and sample spattering, while use of insufficient vacuum may reduce DNA yield and purity.

Table 1. Regulation of vacuum pressure for QIAquick procedures

Procedure	Vacuum manifold	Module used for checking pressure*	Vacuum pressure‡ mbar	Vacuum pressure‡ mm Hg
QIAquick 8	QIAvac 6S	QIAquick 8 Strip(s)†	–200 to –600	–150 to –450
QIAquick 96	QIAvac 96	QIAquick 96 Plate	–100 to –600	–75 to –450

* 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 2. 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 (atmos)	0.000987
Pounds per square inch (psi)	0.0145

Protocol: QIAquick 8 PCR Purification

This protocol is designed for multiple purification of single- or double-stranded PCR products of 100 bp – 10 kb from primers, nucleotides, polymerases, and salts using QIAquick 8 strips on a QIAvac 6S manifold. A protocol for gel extraction in 8-well format is available from your local QIAGEN Technical Services Department (see back cover for contact details).

Please read the protocol completely before starting.

Important points before starting

- Add ethanol (96–100%) to Buffer PE concentrate before the first use (see bottle label for volume).
- QIAvac 6S operates with any vacuum source (e.g., a house vacuum, vacuum pump, or water aspirator) that generates negative pressure (vacuum). The QIAquick 8 PCR Purification System should be used with vacuum pressures between –200 to –600 mbar (–150 to –450 mm Hg) when the vacuum is applied to the appropriate number of **empty** QIAquick 8 strips on QIAvac 6S (e.g., if 3 QIAquick strips are to be used, measure the vacuum with 3 **empty** QIAquick strips, see page 16).
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during manipulations.
- Buffer PB contains a guanidine salt and is therefore not compatible with disinfecting reagents containing bleach. See page 6 for safety information. Take appropriate safety measures and wear gloves when handling.
- A reservoir or multichannel pipet facilitates liquid handling.

Procedure

1. **Prepare QIAvac 6S and QIAquick 8-well strips:**

   (**Note**: the following procedure applies to the manifold with a hinged lid and spring lock.)

   Open QIAvac 6S lid and place QIAquick 8-well strips in the slots of the QIAvac top plate, making sure the strips are seated tightly. Seal any unused slots with blanks (provided with QIAvac), and close QIAvac 6S lid. Place the waste tray inside the QIAvac base, and place the top plate squarely over the base. Seal unused wells of the QIAquick strips with the caps provided.

2. **Add 5 volumes of Buffer PB to 1 volume of the PCR reaction and mix. It is not necessary to remove mineral oil or kerosene. Apply the samples to the wells of the QIAquick strips. Switch on vacuum source.**

   For example, add 500 µl of Buffer PB to 100 µl PCR sample (excluding oil volume).

   Note: Alternatively, if PCR sample volume is ≥100 µl, place QIAquick strips onto the strip holder (provided with QIAvac 6S) and load 5 volumes of Buffer PB into each well of QIAquick strips. (Some Buffer PB will be soaked into the membrane but the remaining buffer is sufficient to provide correct pH and salt conditions). Then add PCR sample to wells and mix by shaking. After mixing, open QIAvac 6S lid and place QIAquick strips in the slots of the QIAvac top plate, making sure the strips are seated tightly. Close QIAvac 6S lid, seal unused wells of the QIAquick strips with the caps provided, and switch on vacuum source.

3. **After all liquid has passed through the membrane, switch off vacuum. Wash wells of QIAquick strips by adding 1 ml of Buffer PE to each well and switch on vacuum source.**

4. **Repeat step 3.**

5. **After Buffer PE in all wells has passed through the membrane, 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 valves or leakage valves if they are used), allowing maximum airflow to go through the wells.

6. **Switch off vacuum source, and ventilate QIAvac 6S slowly. Lift the top plate from the base (not the QIAquick 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 QIAquick strips with clean absorbent paper. Proceed either to step 7a, or 7b, as desired.**

   This step removes residual Buffer PE which may be present around the outlet nozzles and collars of the QIAquick strips. Residual ethanol, which is in Buffer PE, may inhibit subsequent enzymatic reactions, e.g., sequencing.

7a. For elution into provided collection microtubes:

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

7b. For elution into a 96-well microplate:

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

8. To elute DNA, add 80 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or H_2O to the center of each well of the QIAquick strips, incubate for 1 min, and switch on vacuum source for 5 min. Switch off vacuum source and ventilate QIAvac 6S slowly. Alternatively, for increased DNA concentration, use 60 µl elution buffer.

Important: Ensure that the elution buffer is dispensed directly onto the center of QIAquick membrane for complete elution of bound DNA. Please note that the average eluate volume is 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume.

If using water for elution, we recommend using the RNase-free water provided in the QIAquick 96 PCR Purification Kit. If water from other sources is used, ensure that the pH is between pH 7.0 and pH 8.5.

Protocol: QIAquick 96 PCR Purification

This protocol is designed for high-throughput purification of single- or double-stranded PCR products of 100 bp – 10 kb from primers, nucleotides, polymerases, and salts using QIAquick 96 plates on a QIAvac 96 manifold. A protocol for gel extraction in 96-well format is available from your local QIAGEN Technical Services Department (see back cover for contact details).

Please read the protocol completely before starting.

Important points before starting

- Add ethanol (96–100%) to Buffer PE concentrate before first use (see bottle label for volume).
- QIAvac 96 operates with any vacuum source (e.g., a house vacuum, vacuum pump, or water aspirator) that generates negative pressure (vacuum). The QIAquick 96 PCR Purification System should be used with vacuum pressures between –100 to –600 mbar (–60 to –450 mm Hg) when the vacuum is applied to an **empty** QIAquick 96 plate on the QIAvac 96 (see page 16).
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during manipulations.
- Buffer PM contains a guanidine salt and is therefore not compatible with disinfecting reagents containing bleach. See page 6 for safety information. Take appropriate safety measures and wear gloves when handling.
- A reservoir or multichannel pipet facilitates liquid handling.

Procedure

1. **Prepare QIAvac 96 and QIAquick 96-well plate:**

   Place waste tray inside QIAvac base, and place QIAvac top plate squarely over the base. Attach QIAvac to a vacuum source. Seal unused wells of QIAquick 96-well plate with tape, and place QIAquick plate in the QIAvac top plate, making sure that the plate is seated securely.

2. **Add 3 volumes of Buffer PM to 1 volume of the PCR sample and mix. It is not necessary to remove mineral oil or kerosene. Apply the samples to the wells of the QIAquick plate. Switch on vacuum source.**

   For example, add 300 µl of Buffer PM to 100 µl PCR sample (excluding oil volume).

   Note: For mixture volumes larger than the capacity of the PCR tubes, transfer PCR samples to wells of a 96-square-well block (see ordering information on page 28), and add Buffer PM. After mixing, apply the samples to the wells of the QIAquick plate and switch on the vacuum.

3. **After all liquid has passed through the membrane, switch off the vacuum source. Wash wells of QIAquick plate by adding 900 µl of Buffer PE to each well and switch on vacuum source.**

4. **Repeat step 3.**

5. **After Buffer PE in all wells has passed through the membrane, 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 valves or leakage valves if they are used), allowing maximum airflow to go through the wells.

6. **Switch off vacuum source, ventilate QIAvac 96 slowly. Lift the top plate from the base (not the QIAquick 96 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 QIAquick 96 plate with clean absorbent paper. Proceed either to step 7a, or 7b, as desired.**

   This step removes residual Buffer PE from around the outlet nozzles and collars of the QIAquick plate. Residual ethanol, which is in Buffer PE, may inhibit subsequent enzymatic reactions, e.g., sequencing.

7a. **For elution into provided collection microtubes:**

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

7b. **For elution into a 96-well microplate:**

   Replace waste tray with empty blue collection microtube rack and place a 96-well microplate directly on the rack. Place the top plate back on base.

8. **To elute, add 80 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or RNase-free water (provided) to the center of each well of the QIAquick 96 plate, incubate for 1 min, and switch on vacuum source for 5 min. Switch off vacuum source and ventilate QIAvac 96 slowly. Alternatively, for increased DNA concentration, use 60 µl elution buffer.**

   Important: Ensure that the elution buffer is dispensed directly onto the center of QIAquick membrane for complete elution of bound DNA. Please note that the average eluate volume is 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer volume.

   If using water for elution, we recommend using the RNase-free water provided in the QIAquick 96 PCR Purification Kit. If water from other sources is used, ensure that the pH is between pH 7.0 and pH 8.5.

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 protocols in this handbook or molecular biology applications (see back cover for contact information).

		Comments and suggestions
Low or no recovery		
a)	Buffer PE did not contain ethanol	Ethanol must be added to Buffer PE concentrate before use. Repeat procedure with correctly prepared Buffer PE.
b)	Inappropriate elution buffer	DNA will only be eluted efficiently in the presence of low salt and pH ≥ 7.0. Use the provided Buffer EB (10 mM Tris·Cl, pH 8.5), or water for elution. See "Elution in low-salt solutions", page 12.
c)	Elution buffer incorrectly dispensed	Dispense elution buffer into the center of each QIAquick well to ensure that the membrane is completely covered with elution buffer.
d)	Insufficient vacuum	Symptoms of insufficient vacuum include eluate volume being less than 60 µl from 80 µl elution buffer, or less than 40 µl from 60 µl elution buffer, or eluate volume varying between wells. Use a stronger vacuum source.
DNA does not perform well in downstream applications		
a)	Salt concentration in eluate too high	Ensure that the two wash steps (steps 3 and 4) are carried out before elution to remove salt.
b)	Eluate contains residual ethanol (samples float out of wells of agarose gel)	Carefully and precisely follow steps 5 and 6 in the protocols, to remove residual ethanol).
c)	Eluate contains primer-dimers	Primer-dimers formed are longer than 20 bp, and are not completely removed. After the binding step, wash the QIAquick wells with 750 µl of 35% guanidine hydrochloride aqueous solution* (35 g in 100 ml). Continue with the Buffer PE wash and the elution step as described in the protocol.

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

		Comments and suggestions
d)	Eluate contains denatured single-stranded DNA, which appears as a smaller smeared band on an analytical gel	The denatured DNA can be renatured before performing enzymatic reactions. Prepare reaction mixture without adding enzyme, incubate the mixture at 95°C for 2 min, and allow the tube to cool slowly to room temperature before adding the enzyme and proceeding. Alternatively, the DNA can be eluted in Buffer EB plus 10 mM NaCl.* The salt and buffering agent promote the renaturation of DNA strands. However the salt concentration of the eluate must then be considered for subsequent applications.

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

Appendix: BioRobot Systems

QIAGEN BioRobot Systems are versatile liquid-handling workstations designed to automate purification of nucleic acids and preparation of enzymatic reactions. Nucleic acid purification on BioRobot Systems combines high throughput with proven QIAGEN technologies to give pure DNA for applications, such as automated fluorescent or manual sequencing, transfection, and enzymatic reactions.

BioRobot Systems are supplied with ready-to-run, tested protocols for high-speed template purification with minimal hands-on time. Plasmid purification, DNA cleanup, and enzymatic-reaction setup (e.g., dye terminator or dye primer sequencing reactions, restriction digests, and PCR) are automatable on BioRobot Systems (Table 3). The BioRobot Pipetting Systems offer high-throughput nucleic acid preparation with short runtimes, reliable pipetting and reaction setup. Advanced electronics, hardware, and software give precision and reliability. An open design allows new applications to be incorporated or protocols to be customized by point-and-click programming. Chemistry, hardware, software, and technical support are all provided by QIAGEN for a complete automated DNA purification system. For detailed specifications and ordering information, please call QIAGEN.*

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

Table 3. 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		✓	✓	✓

* Please note that 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.

Ordering Information

Product	Contents	Cat. no.
QIAquick 8 PCR Purification Kit (50)*	For purification of 50 x 8 PCR reactions: 50 QIAquick 8 Strips, Buffers, Collection Microtubes (1.2 ml), Caps	28144
QIAquick 96 PCR Purification Kit (4)†‡	For purification of 4 x 96 PCR reactions: 4 QIAquick 96 Plates, Buffers, Collection Microtubes (1.2 ml), Caps	28181
QIAquick 96 PCR Purification Kit (24)†	For purification of 24 x 96 PCR reactions: 24 QIAquick 96 Plates, Buffers, Collection Microtubes (1.2 ml), Caps	28183
Related Products — Microspin-Column Format		
QIAquick PCR Purification Kit (50)	For purification of 50 PCR reactions: 50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28104
QIAquick PCR Purification Kit (250)	For purification of 250 PCR reactions: 250 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28106
QIAquick Nucleotide Removal Kit (50)	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28304
QIAquick Nucleotide Removal Kit (250)	250 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28306
QIAquick Gel Extraction Kit (50)	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28704
QIAquick Gel Extraction Kit (250)	250 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28706

* Requires use of QIAvac 6S.

† Requires use of QIAvac 96.

‡ Larger kit sizes available; please inquire.

Ordering Information

Product	Contents	Cat. no.
Individual Buffers		
Buffer PB (500 ml)	500 ml Binding Buffer	19066
Buffer PE (concentrate, 100 ml)	100 ml Wash Buffer (5x concentrate for 500 ml buffer)	19065
Buffer PM (500 ml)	500 ml Binding Buffer	19083
QIAquick BioRobot Kits		
QIAquick 96 PCR BioRobot Kit (4)*	For purification of 4 x 96 PCR products: 4 QIAquick 96 Plates, Reagents, Buffers, Collection Microtubes (1.2 ml) and Caps, 96-Well Microplates RB and Lids, Tape Pads	963141
QIAvac Manifolds and Accessories		
QIAvac 6S	Vacuum manifold for processing 1–24 QIAGEN spin columns or 1–6 QIAGEN 8-well strips: includes QIAvac 6S Top Plate with flip-up lid, Base, Waste Tray, Blanks, Strip Holder, Rack of Collection Microtubes (1.2 ml)	19503
QIAvac 96	Vacuum manifold for processing QIAGEN 96-well plates: includes QIAvac 96 Top Plate, Base, Waste Tray, Plate Holder, Rack of Collection Microtubes (1.2 ml)	19504
Vacuum Regulator	For use with QIAvac manifolds	19530
Square-Well Blocks (24)	96-well blocks with 2.2 ml wells, 24 per case	19573

* Larger kit sizes available; please inquire.

Notes

Notes

QIAGEN Companies

Please see the back cover for contact information for your local QIAGEN office.

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