

QIAquick® Spin Handbook

For

QIAquick PCR Purification Kit

QIAquick Nucleotide Removal Kit

QIAquick Gel Extraction Kit

July 2002



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Kit Contents

QIAquick PCR Purification Kits	(50)	(250)
Catalog No.	28104	28106
QIAquick Spin Columns	50	250
Buffer PB*	30 ml	150 ml
Buffer PE (concentrate)	2 x 6 ml	55 ml
Buffer EB	15 ml	15 ml
Collection Tubes (2 ml)	50	250
Handbook	1	1
QIAquick Nucleotide Removal Kits	(50)	(250)
Catalog No.	28304	28306
QIAquick Spin Columns	50	250
Buffer PN*	30 ml	140 ml
Buffer PE (concentrate)	2 x 6 ml	55 ml
Buffer EB	15 ml	55 ml
Collection Tubes (2 ml)	100	500
Handbook	1	1
QIAquick Gel Extraction Kits	(50)	(250)
Catalog No.	28704	28706
QIAquick Spin Columns	50	250
Buffer QG*	2 x 50 ml	2 x 250 ml
Buffer PE (concentrate)	2 x 10 ml	2 x 50 ml
Buffer EB	15 ml	15 ml
Collection Tubes (2 ml)	50	250
Handbook	1	1

* Buffers PB, PN, and QG contain chaotropic salts which are irritants. Take appropriate laboratory safety measures and wear gloves when handling.

Storage Conditions

QIAquick® Spin Kits should be stored dry at room temperature (15–25°C). Under these conditions, QIAquick Spin Kits can be stored for up to 12 months without showing any reduction in performance and quality. For longer storage, QIAquick Spin Kits can also be stored 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 Spin 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 Spin Kits is monitored routinely and on a lot-to-lot basis. QIAquick Spin Kits are tested by isolation of DNA fragments of various sizes from either aqueous solution or agarose gel. The quality of the isolated DNA is checked by several assays commonly used for nucleic acids. The DNA binding capacity of QIAquick spin columns is tested by determining the recovery from a specific amount of loaded DNA.

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 problems regarding any aspect of QIAquick Spin 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 listed on the last page.

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 contains guanidine hydrochloride, which can form highly reactive compounds when combined with bleach.

In case liquid containing this buffer 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 System.

Buffer PB

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

Buffer PN

Contains sodium perchlorate and isopropanol: harmful, highly flammable. Risk and safety phrases*: R11-22. S13-16-23-26-36-46

Buffer QG

Contains guanidine thiocyanate: harmful. Risk and safety phrases*: R20/21/22-32. 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. R11: Highly Flammable. R22: Harmful if swallowed. R20/21/22: Harmful by inhalation, in contact with skin and if swallowed. R32: Contact with acids liberates very toxic gas. R36/38: Irritating to eyes and skin. S13: Keep away from food, drink and animal feedingstuffs. S16: Explosive when mixed with oxidizing substances. S23: Do not breathe vapour/spray. 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 the container or label.

Introduction

The QIAquick system, designed for rapid DNA cleanup, includes:

- **QIAquick PCR Purification Kits** for direct purification of double- or single-stranded PCR products (100 bp – 10 kb) from amplification reactions and DNA cleanup from other enzymatic reactions.
- **QIAquick Nucleotide Removal Kits** for general cleanup of oligonucleotides and DNA up to 10 kb from enzymatic reactions (e.g. labeling, dephosphorylation, restriction, and tailing).
- **QIAquick Gel Extraction Kits** for extraction of DNA fragments (70 bp – 10 kb) from standard, or low-melt agarose gels in TAE (Tris-acetate/EDTA) or TBE (Tris-borate/EDTA) buffer and DNA cleanup from enzymatic reactions.

QIAquick PCR Kits are also available in multiwell format for preparation of 8 to 96 samples (see page 33 for ordering information).

QIAquick Kits provide high yields of pure nucleic acids, for direct use in applications such as:

- Fluorescent and radioactive sequencing
- Restriction
- Labeling
- Hybridization
- Ligation and transformation
- Amplification
- In vitro transcription
- Microinjection

Enzymatic reaction cleanup using QIAquick Kits

The QIAquick system is suitable for fast cleanup of up to 10 µg of DNA fragments from enzymatic reactions and agarose gels (see the QIAquick DNA Cleanup Guide, page 8). Enzyme contamination of DNA samples can interfere with subsequent downstream applications. QIAquick Spin Kits can be used for highly efficient removal of a broad spectrum of enzymes widely used in molecular biology. In addition, QIAGEN offers the new MinElute™ Reaction Cleanup Kit, which is specially designed for fast and easy DNA cleanup from all enzymatic reactions. Using proven microspin technology, the MinElute Reaction Cleanup Kit delivers highly concentrated purified DNA by using an elution volume of only 10 µl (see ordering information, page 33).

QIAquick DNA Cleanup Guide

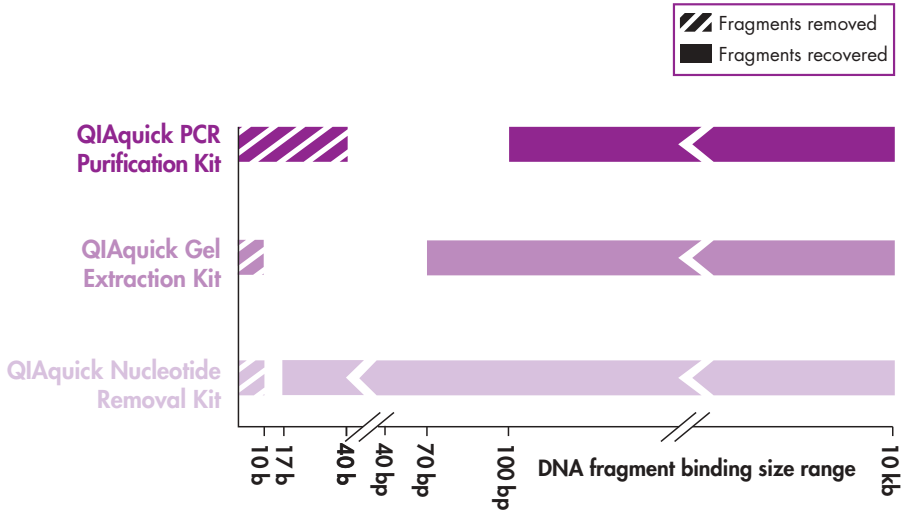
QIAquick Kits can be used to cleanup the following enzymatic reactions:

	From solutions			From gels
	QIAquick PCR Purification Kit	QIAquick Nucleotide Removal Kit	QIAquick Gel Extraction Kit	QIAquick Gel Extraction Kit
Alkaline phosphatase	YES	YES	YES	YES
cDNA synthesis	YES	no	no	YES
DNase, nuclease digestion	YES	YES	YES	YES
Kinase:				
DNA fragments	YES	YES	YES	YES
Oligonucleotides	no	YES	no	no
Ligation	YES	YES	YES	YES
Nick translation	YES	YES	YES	YES
PCR	YES	no	no	YES
Random priming	YES	YES	YES	YES
Restriction digestion	YES	YES	YES	YES
Tailing:				
DNA fragments	YES	YES	YES	YES
Oligonucleotides	no	YES	no	no

Specifications

	QIAquick PCR Purification Kit	QIAquick Nucleotide Removal Kit	QIAquick Gel Extraction Kit
Maximum binding capacity:	10 µg	10 µg	10 µg
Maximum weight of gel slice:	—	—	400 mg
Minimum elution volume:	30 µl	30 µl	30 µl
Capacity of column reservoir:	800 µl	800 µl	800 µl
Typical recoveries			
Recovery of DNA:	90–95% (100 bp – 10 kb)	80–95% (40 bp – 10 kb)	70–80% (70 bp – 10 kb)
Recovery of oligonucleotides (17–40mers):	0	60–80%	10–20%
Recovered:			
Oligonucleotides	—	17–40mers	—
dsDNA	100 bp – 10 kb	40 bp – 10 kb	70 bp – 10 kb
Removed:			
<10mers	YES	YES	YES
17–40mers	YES	no	no

DNA Fragment Binding-Size Range



Recoveries of DNA fragments in the size range between "removed" and "recovered" are not defined.

The QIAquick Principle

The QIAquick system combines the convenience of spin-column technology with the selective binding properties of a uniquely-designed silica-gel membrane. Special buffers provided with each kit are optimized for efficient recovery of DNA and removal of contaminants in each specific application. DNA adsorbs to the silica-membrane in the presence of high salt while contaminants pass through the column. Impurities are efficiently washed away, and the pure DNA is eluted with Tris buffer or water (see page 17). The QIAquick spin columns offer two handling options — as an alternative to processing the spin columns in a microcentrifuge, they can now also be used on any commercial vacuum manifold with luer connectors, e.g., QIAvac 6S or QIAvac 24 with QIAvac Luer Adapters.

Adsorption to QIAquick membrane — salt & pH dependence

The QIAquick silica-gel membrane is uniquely adapted to isolate DNA from both aqueous solutions and agarose gels, and up to 10 µg DNA can bind to each QIAquick column. The binding buffers in QIAquick Spin Kits provide 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 salts (1), which modify the structure of water (2).

Adsorption of DNA to silica also depends on pH. Adsorption 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 pH for DNA binding can be obtained by adding a small volume of 3 M sodium acetate, pH 5.0.

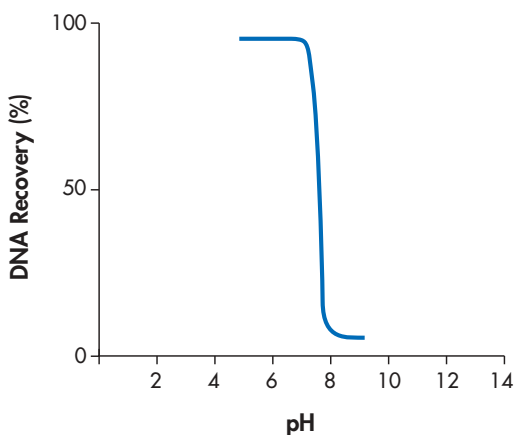


Figure 1. pH dependence of DNA adsorption to silica. 1 µg of a 2.9 kb DNA fragment was adsorbed at different pHs and eluted with Buffer EB (10 mM Tris-Cl, pH 8.5). The graph shows the percentage of DNA recovery, reflecting the relative adsorption efficiency, versus pH of adsorption.

Optimized binding buffers for every DNA cleanup task

All QIAquick Spin Kits contain identical QIAquick spin columns but different binding buffers optimized for each specific application:

- Buffer PB in the QIAquick PCR Purification Kit allows the efficient binding of single- or double-stranded PCR products as small as 100 bp and the quantitative (99.5%) removal of primers up to 40 nucleotides. This kit can therefore be used to remove oligo-dT primers after cDNA synthesis or to remove unwanted linkers in cloning experiments.
- Buffer PN in the QIAquick Nucleotide Removal Kit promotes the adsorption of both oligonucleotides ≥ 17 bases and DNA fragments up to 10 kb to the membrane.
- Buffer QG in the QIAquick Gel Extraction Kit solubilizes the agarose gel slice and provides the appropriate conditions for binding of DNA to the silica membrane.

All of these buffers are also available separately (see ordering information, page 33).

pH indicator in solubilization and binding buffer QG

The binding and solubilization buffer QG has been specially optimized for use with the QIAquick silica-gel membrane. Please note that Buffer QG should not be used with QIAEX II silica resin. Buffer QG contains a pH indicator, allowing easy determination of the optimal pH for DNA binding. DNA adsorption requires a pH ≤ 7.5 , and the pH indicator in Buffer QG appears yellow in this range. If the pH is >7.5 , which can occur if the agarose gel electrophoresis buffer is frequently used or incorrectly prepared, the binding mixture turns orange or violet (Figure 2). This means that the pH of the sample exceeds the buffering capacity of Buffer QG and DNA adsorption will be inefficient. In this case, the pH of the binding mixture can easily be corrected by addition of a small volume of 3 M sodium acetate, pH 5.0, before proceeding with the protocol.

In addition, the color of the binding mixture allows easy visualization of any unsolubilized agarose, ensuring complete solubilization and maximum yields. The indicator dye does not interfere with DNA binding and is completely removed during the cleanup procedure.

Buffer QG does not contain NaI. Residual NaI may be difficult to remove from DNA samples, and reduces the efficiency of subsequent enzymatic reactions such as blunt-end ligation.

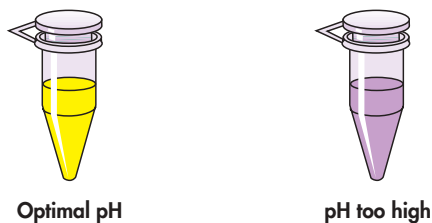


Figure 2. Indicator dye in solubilization and binding Buffer QG identifies optimal pH for DNA binding.

Washing

During the DNA adsorption step, unwanted primers and impurities, such as salts, enzymes, unincorporated nucleotides, agarose, dyes, ethidium bromide, oils, and detergents (e.g., DMSO, Tween® 20) do not bind to the silica membrane, but flow through the column. 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 an additional centrifugation step.

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 eluted with 50 or 30 µl of the provided Buffer EB (10 mM Tris-Cl, pH 8.5), or water. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water to elute, make sure that the pH is within this range. In addition, DNA must be stored at -20°C when eluted with water since DNA may degrade in the absence of a buffering agent. 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.

DNA yield and concentration

DNA yield depends on the following three factors: the volume of elution buffer, how the buffer is applied to the column, and the incubation time of the buffer on the column. 100–200 µl of elution buffer completely covers the QIAquick membrane, ensuring maximum yield, even when not applied directly to the center of the membrane. Elution with ≤50 µl requires the buffer to be added directly to the center of the membrane, and if elution is done with the minimum recommended volume of 30 µl, an additional 1 min incubation is required for optimal yield. DNA will be up to 1.7 times more concentrated if the QIAquick column is incubated for 1 min with 30 µl of elution buffer, than if it is eluted in 50 µl without incubation (Figure 3, page 14).

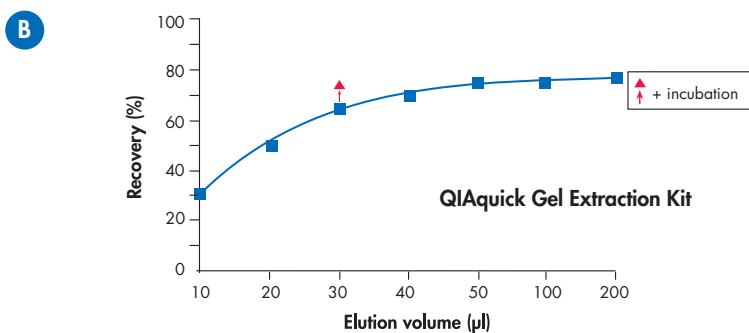
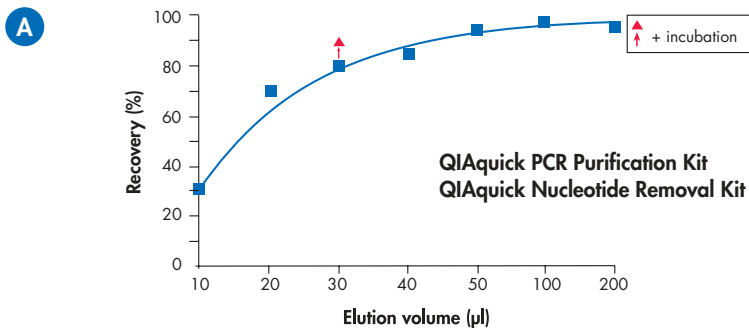


Figure 3. Effect of elution buffer volume on DNA yield for **A** the QIAquick PCR Purification and QIAquick Nucleotide Removal Kit; **B** the QIAquick Gel Extraction Kit. 5 μg of a 2.9 kb DNA fragment were purified and eluted with the indicated volumes of Buffer EB. 30 μl plus 1 minute incubation on the QIAquick column gives DNA yields similar to 50 μl without incubation, but at a concentration 1.7 times greater.

Agarose gel analysis of yield

Yields of DNA following cleanup can be determined by agarose gel analysis. Table 1 shows the total yield obtained following extraction of 1 µg or 0.5 µg starting DNA from an agarose gel with a recovery of 80% or 60% using the QIAquick Gel Extraction Kit. The corresponding amount of DNA in a 1 µl aliquot from 50 µl eluate is indicated. Quantities of DNA fragment corresponding to these 1 µl aliquots are shown on the agarose gel in Figure 4.

Table 1. Amount of DNA in 1 µl aliquots of a 50 µl eluate following QIAquick purification

Starting DNA	Recovery	Total yield (50 µl eluate)	Amount of DNA in 1 µl
1 µg	80%	0.8 µg	16 ng
	60%	0.6 µg	12 ng
0.5 µg	80%	0.4 µg	8 ng
	60%	0.3 µg	6 ng



Figure 4. Quantities of purified 2.7 kb DNA fragment corresponding to 1/50 of the DNA obtained following purification from 1 µg or 0.5 µg starting DNA with a recovery of 80% or 60% (see Table 1). Samples were run on a 1% TAE agarose gel. **M:** lambda-EcoRI-HindIII markers.

Quantitation of DNA fragments

DNA fragments can be quantitated by running a sample alongside standards containing known quantities of the same-sized DNA fragment. The amount of sample DNA loaded can be estimated by visual comparison of the band intensity with that of the standards (Figure 5).

Agarose Gel Analysis

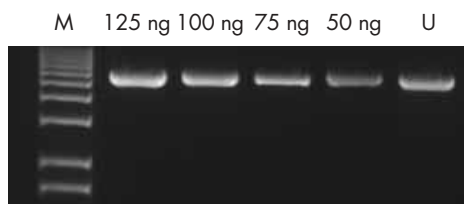


Figure 5. An unknown amount of a 5.5 kb DNA fragment (**U**) was run alongside known quantities (as indicated in ng) of the same DNA fragment. The unknown sample contained 75–100 ng DNA, as estimated by visual comparison with the standards. **M:** 1 kb DNA ladder.

Applications using QIAquick purified DNA

DNA purified with QIAquick is suitable for any subsequent application, such as restriction, labeling, hybridization, PCR, ligation and transformation, radioactive and fluorescent sequencing, in vitro transcription, or microinjection. For direct sequencing of PCR products, refer to chapter 15.2 of *Current Protocols in Molecular Biology*, Ausubel, F. M. et al., eds (1991) Wiley Interscience, New York.

A critical parameter in sequencing reactions is the template-to-primer ratio. Optimal ratios for radioactive sequencing of PCR fragments are provided in Table 2.

Table 2. Optimal molar template-to-primer ratios for manual radioactive sequencing

	Template:primer
Internal primer	1:1 – 1:5
PCR primer	1:10 – 1:20

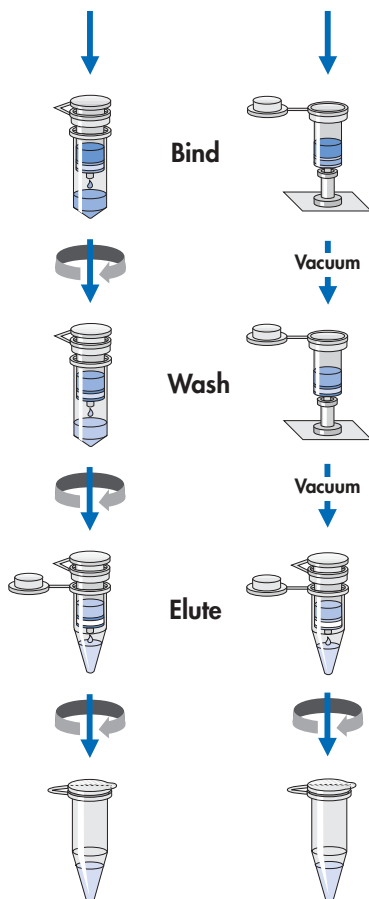
For automated sequencing, the template-to-primer ratio is also important. Please refer to the manufacturer's instructions provided with your sequencing kit.

References

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.

The QIAquick Spin Purification Procedure

PCR reaction
or
Solubilized gel slice
or
Enzymatic reaction



Pure DNA Fragment

QIAquick PCR Purification Kit Protocol

using a microcentrifuge

This protocol is designed to purify single- or double-stranded DNA fragments from PCR and other enzymatic reactions (see page 8). For cleanup of other enzymatic reactions, follow the protocol as described for PCR samples or use the new MinElute Reaction Cleanup Kit. Fragments ranging from 100 bp to 10 kb are purified from primers, nucleotides, polymerases, and salts using QIAquick spin columns in a microcentrifuge.

Notes:

- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifuge steps are at 13,000 rpm (~17,900 x g) in a conventional tabletop microcentrifuge.

- 1. Add 5 volumes of Buffer PB to 1 volume of the PCR sample and mix. It is not necessary to remove mineral oil or kerosene.**

For example, add 500 µl of Buffer PB to 100 µl PCR sample (not including oil).

- 2. Place a QIAquick spin column in a provided 2 ml collection tube.**
- 3. To bind DNA, apply the sample to the QIAquick column and centrifuge for 30–60 s.**
- 4. Discard flow-through. Place the QIAquick column back into the same tube.**
Collection tubes are re-used to reduce plastic waste.
- 5. To wash, add 0.75 ml Buffer PE to the QIAquick column and centrifuge for 30–60 s.**
- 6. Discard flow-through and place the QIAquick column back in the same tube. Centrifuge the column for an additional 1 min.**

IMPORTANT: Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.

- 7. Place QIAquick column in a clean 1.5 ml microcentrifuge tube.**
- 8. To elute DNA, add 50 µl Buffer EB (10 mM Tris-Cl, pH 8.5) or H₂O to the center of the QIAquick membrane and centrifuge the column for 1 min. Alternatively, for increased DNA concentration, add 30 µl elution buffer to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge.**

IMPORTANT: Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA. The average eluate volume is 48 µl from 50 µl elution buffer volume, and 28 µl from 30 µl elution buffer.

Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

QIAquick PCR Purification Kit Protocol

using a vacuum manifold

QIAquick spin columns can now be used on any vacuum manifold with luer connectors, e.g., QIAvac 6S or QIAvac 24 with Luer Adapters. The following protocol is designed to purify single- or double-stranded DNA fragments from PCR and other enzymatic reactions (see page 8). For cleanup of other enzymatic reactions, follow the protocol as described for PCR samples or use the new MinElute Reaction Cleanup Kit. Fragments ranging from 100 bp to 10 kb are purified from primers, nucleotides, polymerases and salts using vacuum-driven sample processing.

- Notes:**
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
 - Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during manipulations.

1. Add 5 volumes of Buffer PB to 1 volume of the PCR sample and mix. It is not necessary to remove mineral oil or kerosene.

For example, add 500 µl of Buffer PB to 100 µl PCR sample (not including oil).

2. Prepare the vacuum manifold and QIAquick columns:

QIAvac 24 (see page 30, and Figure 6):

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

QIAvac 6S manifold (see page 30, and Figure 7):

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

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

3. To bind DNA, load the samples into the QIAquick columns by decanting or pipetting, and apply vacuum. After the samples have passed through the column, switch off the vacuum source.

The maximum loading volume of the column is 800 µl. For sample volumes greater than 800 µl simply load again.

4. To wash, add 0.75 ml of Buffer PE to each QIAquick column and apply vacuum.
5. Transfer each QIAquick column to a microcentrifuge tube or the provided 2 ml collection tubes. Centrifuge for 1 min at 13,000 rpm (~17,900 x g).

IMPORTANT: This spin is necessary to remove residual ethanol (Buffer PE).

6. Place each QIAquick column into a clean 1.5 ml microcentrifuge tube.
7. To elute DNA, add 50 μ l of Buffer EB (10 mM Tris-Cl, pH 8.5) or H₂O to the center of each QIAquick membrane, and centrifuge the columns for 1 min at 13,000 rpm (~17,900 x g). Alternatively, for increased DNA concentration, add 30 μ l elution buffer to the center of each QIAquick membrane, let the columns stand for 1 min, and then centrifuge.

IMPORTANT: Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA. The average eluate volume is 48 μ l from 50 μ l elution buffer volume, and 28 μ l from 30 μ l elution buffer.

Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at -20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

QIAquick Nucleotide Removal Kit Protocol

using a microcentrifuge

This protocol is designed for cleanup of radioactive-, biotin-, or DIG-labeled DNA fragments and oligonucleotides ≥ 17 nucleotides from enzymatic reactions (see page 8). The protocol ensures removal of primers < 10 bases, enzymes, salts, and unincorporated nucleotides. It is possible to use this kit with a vacuum manifold as well as with a microcentrifuge, and a protocol for vacuum processing is available on request from QIAGEN Technical Services or your local distributor. However, we do not recommend processing radioactive samples with a vacuum manifold.

- Notes:**
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
 - All centrifugation steps are in a conventional tabletop microcentrifuge.

1. Add 10 volumes of Buffer PN to 1 volume of the reaction sample and mix.

For example, add 500 μ l Buffer PN to a 50 μ l reaction sample. For DNA fragments ≥ 100 bp, only 5 volumes of Buffer PN are required.

2. Place a QIAquick spin column in a provided 2 ml collection tube.

3. To bind DNA, apply the sample to the QIAquick column and centrifuge for 1 min at 6000 rpm.

4. For radioactive samples:

Place the QIAquick column into a clean 2 ml collection tube and discard the tube containing the radioactive flow-through appropriately.

For non-radioactive samples:

Discard the flow-through and place QIAquick column back into the same tube.

Collection tubes are re-used to reduce plastic waste.

5. For radioactive samples:

To wash QIAquick column, add 500 μ l of Buffer PE and centrifuge for 1 min at 6000 rpm. Discard the flow-through appropriately and repeat wash with another 500 μ l of Buffer PE.

For non-radioactive samples:

To wash QIAquick column, add 750 μ l of Buffer PE and centrifuge for 1 min at 6000 rpm.

6. Discard the flow-through and place the QIAquick column back in the same tube, which should be empty. Centrifuge for an additional 1 min at 13,000 rpm (~17,900 x g).

IMPORTANT: Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifuge.

7. Place the QIAquick column in a clean 1.5 ml microcentrifuge tube.

- 8. To elute DNA, add 100–200 μl of Buffer EB (10 mM Tris-Cl, pH 8.5) or H_2O to the center of the QIAquick membrane and centrifuge the column for 1 min at 13,000 rpm ($\sim 17,900 \times g$). Alternatively, for increased DNA concentration, add 30–50 μl elution buffer to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge.**

IMPORTANT: Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA.

Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at -20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

QIAquick Gel Extraction Kit Protocol

using a microcentrifuge

This protocol is designed to extract and purify DNA of 70 bp to 10 kb from standard or low-melt agarose gels in TAE or TBE buffer. Up to 400 mg agarose can be processed per spin column. This kit can also be used for DNA cleanup from enzymatic reactions (see page 8). For DNA cleanup from enzymatic reactions using this protocol, add 3 volumes of Buffer QG and 1 volume of isopropanol to the reaction, mix, and proceed with step 6 of the protocol. Alternatively, use the new MinElute Reaction Cleanup Kit.

- Notes:**
- The yellow color of Buffer QG indicates a pH ≤ 7.5 .
 - Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
 - Isopropanol (100%) and a heating block or water bath at 50°C are required.
 - All centrifugation steps are carried out at 13,000 rpm ($\sim 17,900 \times g$) in a conventional table-top microcentrifuge.
 - 3 M sodium acetate, pH 5.0, may be necessary.

1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.

Minimize the size of the gel slice by removing extra agarose.

2. Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer QG to 1 volume of gel (100 mg ~ 100 μ l).

For example, add 300 μ l of Buffer QG to each 100 mg of gel. For >2% agarose gels, add 6 volumes of Buffer QG. The maximum amount of gel slice per QIAquick column is 400 mg; for gel slices >400 mg use more than one QIAquick column.

3. Incubate at 50°C for 10 min (or until the gel slice has completely dissolved). To help dissolve gel, mix by vortexing the tube every 2–3 min during the incubation.

IMPORTANT: Solubilize agarose completely. For >2% gels, increase incubation time.

4. After the gel slice has dissolved completely, check that the color of the mixture is yellow (similar to Buffer QG without dissolved agarose).

If the color of the mixture is orange or violet, add 10 μ l of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.

The adsorption of DNA to the QIAquick membrane is efficient only at pH ≤ 7.5 . Buffer QG contains a pH indicator which is yellow at pH ≤ 7.5 and orange or violet at higher pH, allowing easy determination of the optimal pH for DNA binding.

5. Add 1 gel volume of isopropanol to the sample and mix.

For example, if the agarose gel slice is 100 mg, add 100 μ l isopropanol. This step increases the yield of DNA fragments <500 bp and >4 kb. For DNA fragments between 500 bp and 4 kb, addition of isopropanol has no effect on yield. Do not centrifuge the sample at this stage.

6. **Place a QIAquick spin column in a provided 2 ml collection tube.**
7. **To bind DNA, apply the sample to the QIAquick column, and centrifuge for 1 min.**

The maximum volume of the column reservoir is 800 μ l. For sample volumes of more than 800 μ l, simply load and spin again.
8. **Discard flow-through and place QIAquick column back in the same collection tube.**

Collection tubes are re-used to reduce plastic waste.
9. **(Optional): Add 0.5 ml of Buffer QG to QIAquick column and centrifuge for 1 min.**

This step will remove all traces of agarose. It is only required when the DNA will subsequently be used for direct sequencing, in vitro transcription or microinjection.
10. **To wash, add 0.75 ml of Buffer PE to QIAquick column and centrifuge for 1 min.**

Note: If the DNA will be used for salt sensitive applications, such as blunt-end ligation and direct sequencing, let the column stand 2–5 min after addition of Buffer PE, before centrifuging.
11. **Discard the flow-through and centrifuge the QIAquick column for an additional 1 min at 13,000 rpm (~17,900 \times g).**

IMPORTANT: Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.
12. **Place QIAquick column into a clean 1.5 ml microcentrifuge tube.**
13. **To elute DNA, add 50 μ l of Buffer EB (10 mM Tris-Cl, pH 8.5) or H₂O to the center of the QIAquick membrane and centrifuge the column for 1 min. Alternatively, for increased DNA concentration, add 30 μ l elution buffer to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge for 1 min.**

IMPORTANT: Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA. The average eluate volume is 48 μ l from 50 μ l elution buffer volume, and 28 μ l from 30 μ l.

Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at -20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

QIAquick Gel Extraction Kit Protocol

using a vacuum manifold

QIAquick spin columns can now be used on any vacuum manifold with luer connectors, e.g., QIAvac 6S with Luer Adapters. The following protocol is designed to extract and purify DNA of 70 bp to 10 kb from standard or low-melt agarose gels in TAE or TBE buffer using vacuum-driven processing. Up to 400 mg agarose can be processed per spin column. This kit can also be used for DNA cleanup from enzymatic reactions (see page 8). For DNA cleanup from enzymatic reactions using this protocol, add 3 volumes of Buffer QG and 1 volume of isopropanol to the reaction and mix. Set up the vacuum manifold as described in step 3 and then proceed with step 6 of the protocol. Alternatively, use the new MinElute Reaction Cleanup Kit.

- Notes:**
- The yellow color of Buffer QG indicates a pH ≤ 7.5 .
 - Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
 - Isopropanol (100%) and a heating block or water bath at 50°C are required.
 - Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during manipulations.
 - 3 M sodium acetate, pH 5.0, may be necessary.

1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.

Minimize the size of the gel slice by removing extra agarose.

2. Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer QG to 1 volume of gel (100 mg ~ 100 μ l).

For example, add 300 μ l of Buffer QG to each 100 mg of gel. For >2% agarose gels, add 6 volumes of Buffer QG. The maximum amount of gel slice per QIAquick column is 400 mg; for gel slices >400 mg use more than one QIAquick column.

3. Incubate at 50°C for 10 min (or until the gel slice has completely dissolved). To help dissolve gel, mix by vortexing the tube every 2–3 min during the incubation.

IMPORTANT: Solubilize agarose completely. For >2% gels, increase incubation time.

During the incubation, prepare the vacuum manifold and QIAquick columns:

QIAvac 24 (see page 30, and Figure 6):

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

QIAvac 6S manifold (see page 30, and Figure 7):

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

Other vacuum manifolds: follow the suppliers instructions. Insert each QIAquick-column into a luer connector.

4. After the gel slice has dissolved completely, check that the color of mixture is yellow (similar to Buffer QG without dissolved agarose).

If the color of the sample is orange or violet, add 10 μ l of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.

The adsorption of DNA to the QIAquick membrane is efficient only at pH ≤ 7.5 . Buffer QG contains a pH indicator which is yellow at pH ≤ 7.5 and orange or violet at higher pH, allowing easy determination of the optimal pH for DNA binding.

5. Add 1 gel volume of isopropanol to the sample and mix.

For example, if the agarose gel slice is 100 mg, add 100 μ l isopropanol. This step increases the yield of DNA fragments <500 bp and >4 kb. For DNA fragments between 500 bp and 4 kb, addition of isopropanol has no effect on yield. Do not centrifuge the sample at this stage.

6. To bind DNA, pipet the sample onto the QIAquick column and apply vacuum. After the sample has passed through the column, switch off vacuum source.

The maximum volume of the column reservoir is 800 μ l. For sample volumes of more than 800 μ l, simply load again.

7. (Optional): Add 0.5 ml of Buffer QG to QIAquick column and apply vacuum.

This step will remove all traces of agarose. It is only required when the DNA will subsequently be used for direct sequencing, in vitro transcription or microinjection.

8. To wash, add 0.75 ml of Buffer PE to QIAquick column and apply vacuum.

Note: If the DNA will be used for salt sensitive applications, such as blunt-end ligation and direct sequencing, let the column stand 2–5 min after addition of Buffer PE before applying vacuum.

9. **Transfer QIAquick column to a clean 1.5 ml microcentrifuge tube or to a provided 2 ml collection tube. Centrifuge for 1 min at 13,000 rpm (~17,900 x g).**

IMPORTANT: This spin is necessary to remove residual ethanol (Buffer PE).

10. **Place QIAquick column in a clean 1.5 ml microcentrifuge tube.**

11. **To elute DNA, add 50 μ l of Buffer EB (10 mM Tris-Cl, pH 8.5) or H₂O to the center of the QIAquick membrane and centrifuge the column for 1 min at 13,000 rpm (~17,900 x g). Alternatively, for increased DNA concentration, add 30 μ l elution buffer, let stand for 1 min, and then centrifuge for 1 min.**

IMPORTANT: Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA. The average eluate volume is 48 μ l from 50 μ l elution buffer volume, and 28 μ l from 30 μ l.

Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at -20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

Troubleshooting Guide

This troubleshooting guide 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).

Comments and Suggestions

Low or no recovery

- | | | |
|------------|--|--|
| | <ul style="list-style-type: none">• Buffer PE did not contain ethanol | Ethanol must be added to Buffer PE (concentrate) before use. Repeat procedure with correctly prepared Buffer PE. |
| | <ul style="list-style-type: none">• Inappropriate elution buffer | DNA will only be eluted efficiently in the presence of low-salt buffer (e.g., Buffer EB: 10 mM Tris-Cl, pH 8.5) or water. See "Elution in low-salt solutions", page 13. |
| | <ul style="list-style-type: none">• Elution buffer incorrectly dispensed | Add elution buffer to the center of the QIAquick membrane to ensure that the buffer completely covers the membrane. This is particularly important when using small elution volumes (30 μ l). |
| <i>Gel</i> | <ul style="list-style-type: none">• Gel slice incompletely solubilized | After addition of Buffer QG to the gel slice, mix by vortexing the tube every 2–3 minutes during the 50°C incubation. DNA will remain in any undissolved agarose. |
| <i>Gel</i> | <ul style="list-style-type: none">• pH of electrophoresis buffer too high (binding mixture turns orange or violet) | The electrophoresis buffer has been repeatedly used or incorrectly prepared, resulting in a sample pH that exceeds the buffering capacity of Buffer QG and leads to inefficient DNA binding. Add 10 μ l of 3 M sodium acetate, pH 5.0, to the sample and mix. The color of the mixture will turn yellow indicating the correct pH for DNA binding. Even for binding mixtures with only small color changes (slight orange color), add the 10 μ l sodium acetate. |
| <i>Gel</i> | <ul style="list-style-type: none">• Gel slice was too large (>400 mg) | 70–80% recovery can only be obtained from \leq 400 mg gel slice per QIAquick column. For gel slices >400 mg, use multiple QIAquick columns. |
| <i>Gel</i> | <ul style="list-style-type: none">• Cloudy and gelatinous appearance of sample mixture after addition of isopropanol | This may be due to salt precipitation, and will disappear upon mixing the sample. Alternatively, the gel slice may not be completely solubilized. In this case, apply the mixture to the QIAquick column, centrifuge, and then add 0.5 ml Buffer QG to the column. Let stand for 1 min at room temperature, and then centrifuge and continue with the procedure. This additional wash will solubilize remaining agarose. |

Comments and Suggestions

DNA does not perform well, e.g., in ligation reactions

- Salt concentration in eluate too high Modify the wash step by incubating the column for 5 min at room temperature after adding 750 µl of Buffer PE, then centrifuge.
- Eluate contains residual ethanol Ensure that the wash flow-through is drained from the collection tube and that the QIAquick column is then centrifuged at 13,000 rpm (~17,900 x g) for an additional 1 min.
- Gel* • Eluate contaminated with agarose The gel slice is incompletely solubilized or weighs >400 mg. Repeat procedure, including the optional Buffer QG column-wash step.
- PCR* • Eluate contains primer-dimers Primer-dimers formed are longer than 20 bp, and are not completely removed. After the binding step, wash the QIAquick column with 750 µl of a 35% guanidine hydrochloride aqueous solution (35 g in 100 ml). Continue with the Buffer PE wash step and the elution step as in the protocol.
- Eluate contains denatured ssDNA, which appears as smaller smeared band on an analytical gel Use the eluted DNA to prepare the subsequent enzymatic reaction but omit the enzyme. To reanneal the ssDNA, incubate the reaction mixture at 95°C for 2 min, and allow the tube to cool slowly to room temperature. Add the enzyme and proceed as usual. Alternatively, the DNA can be eluted in 10 mM Tris buffer containing 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.

Gel: refers to QIAquick Gel Extraction Kits only

PCR: refers to QIAquick PCR Purification Kits only

Other notes refer to all kits

Appendix: QIAvac Vacuum Manifolds

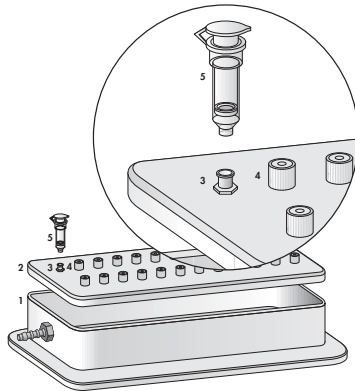


Figure 6. Components of the QIAvac 24 manifold.

- | | |
|---|--|
| <ol style="list-style-type: none"> 1. QIAvac 24 base 2. QIAvac 24 lid 3. Luer extension of QIAvac 24 | <ol style="list-style-type: none"> 4. Luer extension closed with luer cap 5. QIAquick spin column* |
|---|--|

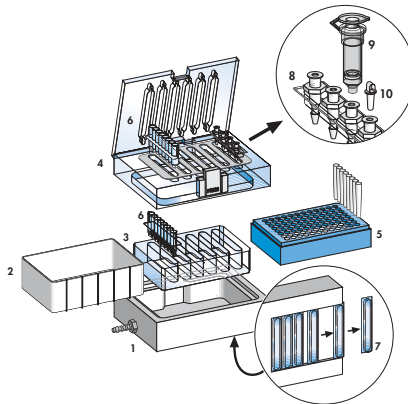


Figure 7. Components of the QIAvac 6S manifold.

- | | |
|--|--|
| <ol style="list-style-type: none"> 1. QIAvac base, which holds a waste tray, a strip holder, or a microtube rack 2. Waste tray 3. QIAvac strip holder to hold 8-well strips 4. QIAvac top plate with slots for 8-well strips or QIAvac Luer Adapters | <ol style="list-style-type: none"> 5. Microtube rack 6. 8-well strip* 7. Blanks to seal unused slots 8. QIAvac Luer Adapter† 9. QIAquick spin column* 10. Plug to seal unused luer connectors† |
|--|--|

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

† Not included with QIAvac 6S. Must be purchased separately.

Handling Guidelines for QIAvac 6S

QIAvac 6S facilitates DNA cleanup with QIAquick by providing a convenient modular vacuum manifold, which, in combination with QIAvac Luer Adapters, allows easy processing of QIAquick spin columns, as an alternative to centrifugation. The following recommendations should be followed when handling the QIAvac 6S vacuum manifold.

- Always store QIAvac 6S vacuum manifold 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 vacuum manifold on a secure bench top or work area. If dropped, the manifold may crack.
- The components of QIAvac manifolds are not resistant to ethanol, methanol, or other organic solvents (Table 4). Do not bring solvents into contact with the vacuum manifold. If solvents are spilled on the unit, rinse thoroughly with distilled water, and do not incubate acrylic components in alcohol-containing reagents for long periods of time. 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 the QIAvac 6S 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.
- Remove blanks from the slots of the top plate after use and store them under the manifold.

Table 4. Chemical Resistance Properties of QIAvac 6S

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 Alcohol	

Handling Guidelines for QIAvac 24

The following guidelines should be followed when working with QIAvac 24.

- Always place QIAvac 24 on a secure bench top or work area. If dropped, the QIAvac 24 manifold may crack.
- Always store QIAvac 24 clean and dry. To clean, simply rinse all components with distilled water and allow to air dry or dry with paper towels.
- The components of QIAvac 24 are not resistant to certain solvents (Table 3). If these solvents are spilled on the unit, rinse it thoroughly with water.
- To ensure consistent performance, do not apply silicone or vacuum grease to any part of the QIAvac 24 manifold.
- Always use caution and wear safety glasses when working near a vacuum manifold under pressure.
- Contact QIAGEN Technical Services or your local distributor for information concerning spare or replacement parts.

Table 3. Chemical resistance properties of QIAvac 24

Resistant to:		Not resistant to:
Chlorine Bleach	Acetone	Phenol
Hydrochloric acid	Chromic acid	Benzene
Sodium chloride	Concentrated alcohols	Chloroform
Sodium hydroxide	Chaotropic salts	Ethers
Urea	SDS	Toluene
Acetic acid	Tween 20	

Trademarks

Patented or patent-pending technology and /or registered or registration-pending trademarks of QIAGEN: QIAGEN[®], QIAquick[®], QIAvac, MinElute[™].

The PCR process is covered by U.S. patents 4,683,195 and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

AmpliQ is a registered trademark of Roche Molecular Systems, Inc.

Tween is a registered trademark of ICI Americas Inc.

Ordering Information

Product	Contents	Cat. No.
QIAquick Spin Kits		
QIAquick PCR Purification Kit (50)	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28104
QIAquick PCR Purification Kit (250)	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
Related Products		
MinElute Reaction Cleanup Kit (50)	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28204
MinElute Reaction Cleanup Kit (250)	250 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28206
MinElute Gel Extraction Kit (50)	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28604
MinElute Gel Extraction Kit (250)	250 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28606
MinElute PCR Purification Kit (50)	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28004
MinElute PCR Purification Kit (250)	250 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28006
QIAquick 8 PCR Purification Kit (10)*	For purification of 10 x 8 PCR reactions: 10 QIAquick 8 Strips, Buffers, Collection Microtubes (1.2 ml) and Caps	28142
QIAquick 8 PCR Purification Kit (50)*	For purification of 50 x 8 PCR reactions: 50 QIAquick 8 Strips, Buffers, Collection Microtubes (1.2 ml) and Caps	28144

* Requires use of QIAvac 6S.

Ordering Information

Product	Contents	Cat. No.
QIAquick 96 PCR Purification Kit (1)*	For purification of 1 x 96 PCR reactions: 1 QIAquick 96 Plate, Buffers, and Collection Microtubes (1.2 ml) and Caps	28180
QIAquick 96 PCR Purification Kit (4)*	For purification of 4 x 96 PCR reactions: 4 QIAquick 96 Plates, Buffers, and Collection Microtubes (1.2 ml) and Caps	28181
Individual Buffers		
Buffer PB	500 ml Binding Buffer	19066
Buffer PN	500 ml Binding Buffer	19071
Buffer PE (concentrate)	100 ml Buffer PE (5x concentrate; final volume 500 ml)	19065
Buffer QG [†] (with pH indicator)	250 ml Solubilization and Binding Buffer (with pH indicator)	19063
QIAvac Manifolds and Accessories		
QIAvac 24	Vacuum manifold for processing 1–24 spin columns: includes QIAvac 24 Base, Lid, Luer Caps	19403
QIAvac 6S	Vacuum manifold for processing 1–6 QIAGEN 8-well strips: includes QIAvac 6S Top Plate with flip-up lid, Base, Waste Tray, Blanks, Strip Holder	19503
QIAvac 96	Vacuum manifold for processing QIAGEN 96 well-plates: includes QIAvac 96 Top plate, Base, Waste Tray, Plate Holder	19504
QIAvac Luer Adapter Set [‡]	For processing 1–24 QIAGEN spin columns on QIAvac 6S: 6 adapters with 4 luer connectors each, 24 plugs	19541
Vacuum Regulator	For use with QIAvac manifolds	19530

* Requires use of QIAvac 96.

[†] Additional Buffer QG may be required for routine purifications from gel slices >300 mg from gels containing >2% agarose.

[‡] Compatible only with QIAvac Top Plates containing flip-up lid.

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

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