

## MinElute<sup>®</sup> Handbook

### MinElute PCR Purification Kit

For purification of PCR products (70 bp to 4 kb)  
in low elution volumes

### MinElute Gel Extraction Kit

For gel extraction of DNA fragments (70 bp to 4 kb)  
in low elution volumes

### MinElute Reaction Cleanup Kit

For cleanup of DNA (70 bp to 4 kb)  
from enzymatic reactions



# QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

## **QIAGEN sets standards in:**

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit [www.qiagen.com](http://www.qiagen.com) .

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## Kit Contents

<b>MinElute PCR Purification Kits</b>	<b>(50)</b>	<b>(250)</b>
<b>Catalog no.</b>	<b>28004</b>	<b>28006</b>
MinElute 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
pH Indicator	800 µl	800 µl
Collection Tubes (2 ml)	50	250
Loading Dye	110 µl	550 µl
Handbook	1	1

<b>MinElute Gel Extraction Kits</b>	<b>(50)</b>	<b>(250)</b>
<b>Catalog no.</b>	<b>28604</b>	<b>28606</b>
MinElute Spin Columns	50	250
Buffer QG*	2 x 50 ml	2 x 250 ml
Buffer PE (concentrate)	2 x 6 ml	55 ml
Buffer EB	15 ml	15 ml
Collection Tubes (2 ml)	50	250
Loading Dye	110 µl	550 µl
Handbook	1	1

<b>MinElute Reaction Cleanup Kits</b>	<b>(50)</b>	<b>(250)</b>
<b>Catalog no.</b>	<b>28204</b>	<b>28206</b>
MinElute Spin Columns	50	250
Buffer ERC*	20 ml	85 ml
Buffer PE	2 x 6 ml	55 ml
Buffer EB	15 ml	15 ml
Collection Tubes (2 ml)	50	250
Loading Dye	110 µl	550 µl
Handbook	1	1

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

## Storage

Upon arrival, open the kit and store MinElute spin columns at 2–8°C. The remaining kit components can be stored at room temperature (15–25°C). Under these conditions, MinElute Kits can be stored for up to 12 months without showing any reduction in performance and quality. Check buffers for precipitate before use and redissolve at 37°C if necessary. The entire kit can be stored at 2–8°C, but in this case buffers should be redissolved before use. Make sure that all buffers are at room temperature when used.

## Product Use Limitations

MinElute 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. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

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

## Quality Control

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

## 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 MinElute Kits, or QIAGEN products in general, please do not hesitate to contact us.

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

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit [www.qiagen.com](http://www.qiagen.com) ).

## 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](http://www.qiagen.com/ts/msds.asp) where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

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

Buffers PB and ERC 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 MinElute DNA Cleanup System

### Buffers PB

Contains guanidine hydrochloride and isopropanol: harmful, irritant, flammable. Risk and safety phrases: \* R10-22-36/38, S23-26-36/37/39-46

### Buffer ERC

Contains guanidine hydrochloride and isopropanol: harmful, irritant, flammable. Risk and safety phrases: \* R10-22-36/38, S23-26-36/37/39-46

### Buffer QG

Contains guanidine thiocyanate: harmful, irritant. 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. 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. 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.

## Product Specifications

	MinElute PCR Purification Kit	MinElute Gel Extraction Kit	MinElute Reaction Cleanup Kit
Maximum binding capacity:	5 µg	5 µg	5 µg
Recovery of DNA:	80% (70 bp – 4kb)	80% (70 bp – 4 kb)	80% (70 bp – 4 kb)
Maximum weight of gel slice:	—	400 mg	—
Elution volume:	10 µl	10 µl	10 µl
Volume of eluate:	9 µl	9 µl	9 µl
Capacity of column reservoir:	800 µl	800 µl	800 µl
<b>Recovered:</b>			
dsDNA	70 bp – 4 kb	70 bp – 4 kb	70 bp – 4 kb
<b>Removed:</b>			
<40mers	<b>YES</b>	n.a.	<b>YES</b>

n.a.: not applicable

## Introduction

The MinElute DNA Cleanup system is specially designed for fast and easy isolation of DNA fragments from PCR reactions, agarose gels, or enzymatic reactions with an extremely small elution volume of only 10  $\mu$ l. Designed for rapid DNA cleanup, with extremely high end-concentration of DNA, the MinElute system includes:

- **MinElute PCR Purification Kits** for direct purification of double-stranded PCR products (70 bp – 4 kb) from amplification reactions.
- **MinElute Gel Extraction Kits** for extraction of DNA fragments (70 bp – 4 kb) from standard or low-melt agarose gels in TAE (Tris-acetate/EDTA) or TBE (Tris-borate/EDTA) buffer.
- **MinElute Reaction Cleanup Kits** to purify double-stranded DNA fragments (70 bp – 4 kb) from all enzymatic reactions.

The MinElute Reaction Cleanup Kit can be used for DNA cleanup from all enzymatic reactions, including:

- Dephosphorylation
- Restriction enzyme digestion
- Ligation
- Primed synthesis
- Endlabeling
- Nick translation

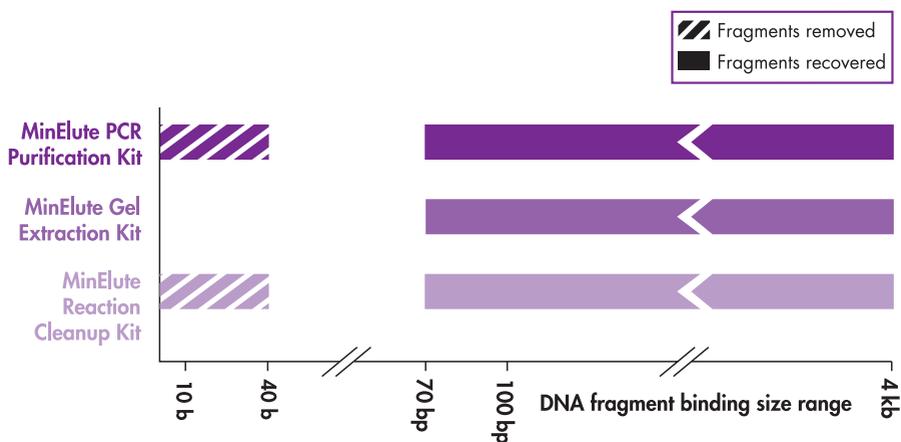
During DNA cleanup using MinElute Reaction Cleanup Kits, all enzymes are removed, independent of size and secondary structure. Table 1 lists examples of enzymes that are used in the above reactions and are removed, together with salts and oligomers, during the MinElute Reaction Cleanup procedure.

**Table 1. Enzymes Commonly Used in Enzymatic Manipulation of DNA**

<b>Protein</b>	<b>Molecular weight per enzyme subunit (kDa)</b>
DNA Polymerase I	109
Klenow fragment	62
Calf intestinal alkaline phosphatase (CIP)	69
T4 DNA ligase	55
T4 Polynucleotide kinase	35
Terminal transferase	32
DNase I	31

MinElute Kits provide high yields of pure nucleic acids in minimal elution volumes. The highly concentrated DNA is suitable for direct use in applications such as:

- Ligation and transformation
- Radioactive and fluorescent sequencing
- Restriction enzyme digestion
- Labeling
- In vitro transcription
- Amplification
- Microinjection
- Hybridization



**Figure. DNA fragment binding-size range.** Recoveries of DNA fragments in the size range between “removed” and “recovered” are not defined.

### Automated DNA cleanup

The MinElute PCR Purification Kit and MinElute Gel Extraction Kit can be fully automated on the QIAcube. The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., bind, wash, and elute) enabling purification of high-quality DNA. The QIAcube is preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids, and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at [www.qiagen.com/MyQIAcube](http://www.qiagen.com/MyQIAcube). A detailed protocol for using QIAquick spin columns on the QIAcube is provided with the QIAcube.

**Note:** It is not necessary to add pH indicator I to Buffer PB when using the QIAcube.

## The MinElute Principle

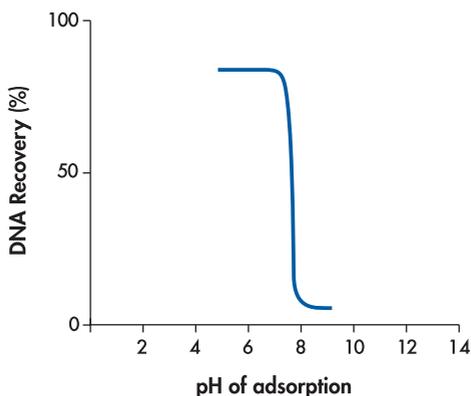
The MinElute system combines the convenience of spin-column technology with the selective binding properties of a uniquely designed silica membrane. MinElute columns are designed to give high end-concentrations of purified DNA fragments for subsequent reactions. Special buffers provided with each kit 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 concentrations of 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). MinElute columns offer two handling options — as an alternative to processing the spin columns in a microcentrifuge, they can also be used on any commercial vacuum manifold with luer connectors (e.g., QIAvac 24 Plus or QIAvac 6S with QIAvac Luer Adapters). The uniquely designed MinElute column with specialized membrane assembly allows purification of concentrated DNA fragments in high yields, in as little as 9  $\mu$ l eluate (Figure 1).



**Figure 2. Unique membrane assembly enables low elution volumes.** Cross-section of MinElute column.

## Adsorption to MinElute membrane — salt and pH dependence

The MinElute silica membrane is uniquely adapted to purify DNA from both aqueous solutions and agarose gels, and up to 5 µg DNA can bind to each MinElute column. The binding buffers in MinElute Spin Kits provide the correct salt concentration and pH for adsorption of DNA to the MinElute membrane. The adsorption of nucleic acids to silica 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 80% if the pH is  $\leq 7.5$ , and is reduced drastically at higher pH (Figure 2). 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 3. pH dependence of DNA adsorption to MinElute membranes** 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

MinElute Kits contain identical MinElute columns and different binding buffers optimized for each specific application:

- Buffer PB in the MinElute PCR Purification Kit allows the efficient binding of double-stranded PCR products as small as 70 bp and the removal of primers up to 40 nucleotides.
- Buffer QG in the MinElute Gel Extraction Kit solubilizes the agarose gel slice and provides the appropriate conditions for binding of DNA to the silica membrane.
- Buffer ERC in the MinElute Reaction Cleanup Kit allows the efficient binding of double stranded DNA as small as 70 bp and the removal of enzymes, salts, and oligomers.
- Buffer PB and Buffer PBI and Buffer QG are also available separately (see Ordering Information).

## pH indicator

Buffers PB, QG, and ERC are specially optimized for use with the MinElute silica membrane. Buffers QG and ERC contain an integrated pH indicator, while an optional pH indicator can be added to Buffer PB allowing easy determination of the optimal pH for DNA binding. DNA adsorption requires a pH  $\leq 7.5$ , and the pH indicator in the buffers will appear yellow in this range. If the pH is  $>7.5$ , which can occur if during agarose gel electrophoresis, the electrophoresis buffer had been used repeatedly or incorrectly prepared, or if the buffer used in an enzymatic reaction is strongly basic and has a high buffering capacity, the binding mixture turns orange or violet (Figure 2). This means that the pH of the sample exceeds the buffering capacity of Buffer PB, QG, or ERC and DNA adsorption will be inefficient. In these cases, 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, in the MinElute Gel Extraction Kit procedure, 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. Buffers PB, QG, and ERC do not contain sodium iodide (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 4. Indicator enables easy checking of the optimal pH.** Indicator dye in solubilization and binding Buffers PBI, QG, and ERC identifies optimal pH for DNA binding.

\* 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) available from the product supplier.

## Washing

During the DNA adsorption step, unwanted primers and impurities, such as salts, enzymes, unincorporated nucleotides, agarose, dyes, ethidium bromide, DMSO, oils, and detergents (e.g., 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 10 µl of the provided Buffer EB (10 mM Tris-Cl, pH 8.5) or water. The volume of the final eluate is 9 µl. 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 buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) is possible, but not recommended because EDTA may inhibit subsequent enzymatic reactions.

## Agarose gel analysis of yield

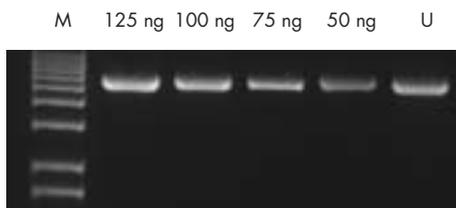
Yields of DNA following cleanup can be analyzed by agarose gel analysis. Table 2 shows the total yield obtained following extraction of 1 µg or 0.5 µg starting DNA from an agarose gel with a recovery of 80% using the MinElute Gel Extraction Kit. The corresponding amount of DNA in a 1 µl aliquot from 10 µl elution volume (9 µl eluate) is indicated.

**Table 2. Amount of DNA in 1 µl Aliquots of a 9 µl Eluate Following MinElute Purification**

Starting DNA	Recovery	Total yield (10 µl elution volume)	Amount of DNA in 1 µl (9 µl eluate)
1 µg	80%	800 ng	89 ng
0.5 µg	80%	400 ng	44 ng

## Quantification of DNA fragments

DNA fragments can be quantified 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 4).



**Figure 5. Agarose gel analysis.** 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.

## Loading dye

Loading dye is provided for analysis of purified DNA samples using electrophoresis. It contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange G) that facilitate estimation of DNA migration distance and optimization of agarose gel run time. Refer to Table 3 to identify the dyes according to migration distance and agarose gel percentage and type. Loading dye is supplied as a 5x concentrate; thus 1 volume of loading dye should be added to 5 volumes of purified DNA.

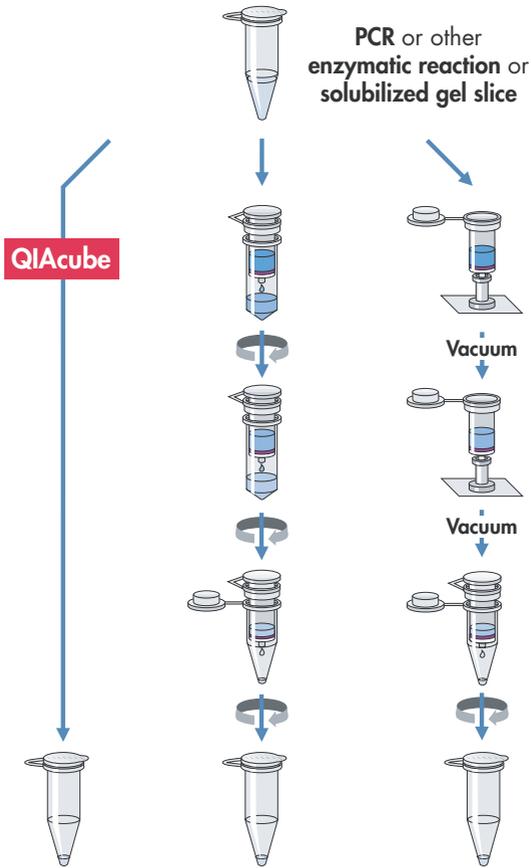
**Table 3. Migration Distance of Gel Tracking Dyes**

%TAE (TBE) agarose gel	Xylene cyanol (light blue)	Bromophenol blue (dark blue)	Orange G (orange)
0.8	5000 bp (3000 bp)	800 bp (400 bp)	150 bp (<100 bp)
1.0	3000 bp (2000 bp)	400 bp (250 bp)	<100 bp (<100 bp)
1.5	1800 bp (1100 bp)	250 bp (100 bp)	<100 bp (<100 bp)
2.0	1000 bp (600 bp)	200 bp (<100 bp)	<100 bp (<100 bp)
2.5	700 bp (400 bp)	100 bp (<50 bp)	<50 bp (<50 bp)

## **Applications using MinElute purified DNA**

DNA purified by the MinElute system is significantly more concentrated than DNA purified by other methods. The highly concentrated DNA makes small reaction volumes possible for downstream applications, leading to increased efficiency (e.g., in ligations). DNA purified with MinElute Kits is suitable for any subsequent application, such as ligation and transformation, radioactive and fluorescent sequencing, restriction enzyme digestion, labeling, hybridization, PCR, *in vitro* transcription, or microinjection.

The MinElute Procedure



Optimal pH



pH too high

# 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, please consult the appropriate material safety data sheets (MSDSs) available from the product supplier.

## For all protocols

- Ethanol (96–100%)\*
- Microcentrifuge
- 1.5 or 2 ml microcentrifuge tubes
- 3 M sodium acetate, pH 5.0, may be necessary for PCR purification and gel extraction protocols.
- Optional: Distilled water or TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH8) for elution of DNA.

## Vacuum protocols

- Vacuum manifold (e.g., QIAvac 24 Plus or QIAvac 6S)
- Vacuum pump (e.g., QIAGEN Vacuum Pump, see ordering information).

## Gel extraction protocols

- Isopropanol (100%)
- Heating block or water bath set at 50°C

\* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

# MinElute PCR Purification Kit Protocol

## using a microcentrifuge

This protocol is designed to purify double-stranded DNA fragments from PCR reactions resulting in high end-concentrations of DNA (see page 12). Fragments ranging from 70 bp to 4 kb are purified from primers, nucleotides, polymerases, and salts using MinElute spin columns in a microcentrifuge.

### Important points before starting

- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are carried out at 17,900 x *g* (13,000 rpm) in a conventional tabletop microcentrifuge at room temperature.
- Add 1:250 volume pH indicator I to Buffer PB (i.e., add 120  $\mu$ l pH indicator I to 30 ml Buffer PB or add 600  $\mu$ l pH indicator I to 150 ml Buffer PB). The yellow color of Buffer PB with pH indicator I indicates a pH of  $\leq 7.5$ .
- Add pH indicator I to entire buffer contents. Do not add pH indicator I to buffer aliquots.
- If the purified PCR product is to be used in sensitive microarray applications, it may be beneficial to use Buffer PB without the addition of pH indicator I.

### Procedure

1. **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.**

For example, add 250  $\mu$ l of Buffer PB to 50  $\mu$ l PCR reaction (not including oil).

2. **If pH indicator I has been added to Buffer PB, check that the color of the mixture is yellow.**

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

3. **Place a MinElute column in a provided 2 ml collection tube in a suitable rack.**
4. **To bind DNA, apply the sample to the MinElute column and centrifuge for 1 min.**  
For maximum recovery, transfer all traces of sample to the column.
5. **Discard flow-through. Place the MinElute column back into the same tube.**
6. **To wash, add 750  $\mu$ l Buffer PE to the MinElute column and centrifuge for 1 min.**
7. **Discard flow-through and place the MinElute column back in the same tube. Centrifuge the column for an additional 1 min at maximum speed.**

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

8. **Place the MinElute column in a clean 1.5 ml microcentrifuge tube.**

9. **To elute DNA, add 10  $\mu$ l Buffer EB (10 mM Tris-Cl, pH 8.5) or water to the center of the 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 center of the membrane for complete elution of bound DNA. The average eluate volume is 9  $\mu$ l from 10  $\mu$ l elution buffer volume.

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  $-20^{\circ}\text{C}$  as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

10. **If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.**

Loading dye contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange G) that facilitate estimation of DNA migration distance and optimization of agarose gel run time. Refer to Table 3 (page 15) to identify the dyes according to migration distance and agarose gel percentage and type.

# MinElute PCR Purification Kit Protocol

## using a vacuum manifold

MinElute spin columns can also be used on any vacuum manifold with luer connectors (e.g., QIAvac 24 Plus or QIAvac 6S with Luer Adapters). The following protocol is designed to purify double-stranded DNA fragments from PCR reactions resulting in high end-concentrations of DNA (see page 12). Fragments ranging from 70 bp to 4 kb are purified from primers, nucleotides, polymerases and salts using vacuum-driven sample processing.

### Important points before starting

- 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.
- Add 1:250 volume pH indicator I to Buffer PB (i.e., add 120  $\mu$ l pH indicator I to 30 ml Buffer PB or add 600  $\mu$ l pH indicator I to 150 ml Buffer PB). The yellow color of Buffer PB with pH indicator I indicates a pH of  $\leq 7.5$ .
- Add pH indicator I to entire buffer contents. Do not add pH indicator I to buffer aliquots.
- If the purified PCR product is to be used in sensitive microarray applications, it may be beneficial to use Buffer PB without the addition of pH indicator I.

### Procedure

1. **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.**

For example, add 250  $\mu$ l of Buffer PB to 50  $\mu$ l PCR reaction (not including oil).

2. **If pH indicator 1 has been added to Buffer PB, check that the color of the mixture is yellow.**

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

3. **Prepare the vacuum manifold and MinElute columns according to step 3a, 3b, or 3c.**

- 3a. **QIAvac 24 Plus (see page 34):**

Insert up to 24 MinElute spin columns into the luer extensions of the QIAvac 24 Plus. Close unused positions with luer caps and connect QIAvac 24 Plus to a vacuum source.

**3b. QIAvac 6S manifold (see page 35):**

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 MinElute 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.

- 3c. Other vacuum manifolds:** follow the supplier's instructions. Insert each MinElute column into a luer connector.
- 4. To bind DNA, load the samples into the MinElute columns by decanting or pipetting, and apply vacuum. After the samples have passed through the columns, switch off the vacuum source.**

For maximum recovery, transfer all traces of sample to the column.

The maximum loading volume of the column is 800  $\mu$ l. For sample volumes greater than 800  $\mu$ l simply load again.

- 5. To wash, add 750  $\mu$ l of Buffer PE to each MinElute column and apply vacuum.**
- 6. Transfer each MinElute column to a microcentrifuge tube or the provided 2 ml collection tubes. Centrifuge for 1 min at  $\geq 10,000 \times g$ .**

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

- 7. Place each MinElute column into a clean 1.5 ml microcentrifuge tube.**
- 8. To elute DNA, add 10  $\mu$ l of Buffer EB (10 mM Tris-Cl, pH 8.5) or water to the center of each membrane, let the columns stand for 1 min, and then centrifuge.**

**IMPORTANT:** Ensure that the elution buffer is dispensed directly onto the center of the membrane for complete elution of bound DNA. The average eluate volume is 9  $\mu$ l from 10  $\mu$ l elution buffer volume.

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  $-20^{\circ}\text{C}$  as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

- 9. If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.**

Loading dye contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange G) that facilitate estimation of DNA migration distance and optimization of agarose gel run time. Refer to Table 3 (page 15) to identify the dyes according to migration distance and agarose gel percentage and type.

# MinElute Gel Extraction Kit Protocol

## using a microcentrifuge

This protocol is designed to extract and purify DNA of 70 bp to 4 kb from standard or low-melt agarose gels in TAE or TBE buffer resulting in high end-concentrations of DNA. Up to 400 mg agarose can be processed per MinElute column.

### Important points before starting

- The yellow color of Buffer QG indicates a pH  $\leq 7.5$ .
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are carried out at  $\geq 10,000 \times g$  in a conventional table-top microcentrifuge at room temperature.

### Procedure

- 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 or approximately 100  $\mu$ l).**  
For example, add 300  $\mu$ 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 spin column is 400 mg; for gel slices >400 mg use more than one MinElute 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).**  
**Note:** If the color of the mixture is orange or violet, add 10  $\mu$ 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 membrane is efficient only at pH  $\leq 7.5$ . Buffer QG contains a pH indicator which is yellow at pH  $\leq 7.5$  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 by inverting the tube several times.**  
For example, if the agarose gel slice is 100 mg, add 100  $\mu$ l isopropanol. Do not centrifuge the sample at this stage.
- 6. Place a MinElute column in a provided 2 ml collection tube in a suitable rack.**

**7. To bind DNA, apply the sample to the MinElute column, and centrifuge for 1 min.**

For maximum recovery, transfer all traces of sample to the column. The maximum volume of the column reservoir is 800  $\mu$ l. For sample volumes of more than 800  $\mu$ l, simply load and spin again.

**8. Discard the flow-through and place the MinElute column back in the same collection tube.****9. Add 500  $\mu$ l of Buffer QG to the spin column and centrifuge for 1 min.****10. Discard the flow-through and place the MinElute column back in the same collection tube.****11. To wash, add 750  $\mu$ l of Buffer PE to the MinElute 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.

**12. Discard the flow-through and centrifuge the MinElute column for an additional 1 min at  $\geq 10,000 \times g$ .**

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

**13. Place the MinElute column into a clean 1.5 ml microcentrifuge tube.****14. To elute DNA, add 10  $\mu$ l of Buffer EB (10 mM Tris-Cl, pH 8.5) or water to the center of the 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 center of the membrane for complete elution of bound DNA. The average eluate volume is 9  $\mu$ l from 10  $\mu$ l elution buffer volume.

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  $-20^{\circ}\text{C}$  as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

**15. If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.**

Loading dye contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange G) that facilitate estimation of DNA migration distance and optimization of agarose gel run time. Refer to Table 3 (page 15) to identify the dyes according to migration distance and agarose gel percentage and type.

# MinElute Gel Extraction Kit Protocol

## using a vacuum manifold

MinElute columns can also be used on any vacuum manifold with luer connectors (e.g., QIAvac 24 Plus or QIAvac 6S with Luer Adapters). The following protocol is designed to extract and purify DNA of 70 bp to 4 kb from standard or low-melt agarose gels in TAE or TBE buffer using vacuum-driven processing resulting in high end-concentrations of DNA. Up to 400 mg agarose can be processed per MinElute column.

### Important points before starting

- The yellow color of Buffer QG indicates a pH  $\leq 7.5$ .
- 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.

### Procedure

- 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 or approximately 100  $\mu$ l).**  
For example, add 300  $\mu$ 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 spin column is 400 mg; for gel slices >400 mg use more than one spin 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. During the incubation, prepare the vacuum manifold and MinElute columns according to steps 4a, 4b, or 4c.**
  - 4a. QIAvac 24 Plus (see page 34):**  
Insert up to 24 MinElute columns into the luer extensions of the QIAvac 24 Plus. Close unused positions with luer caps and connect QIAvac 24 Plus to a vacuum source.

**4b. QIAvac 6S manifold (see page 35):**

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 MinElute 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.

- 4c. Other vacuum manifolds:** follow the suppliers instructions. Insert each MinElute column into a luer connector.
- 5. After the gel slice has dissolved completely, check that the color of mixture is yellow (similar to Buffer QG without dissolved agarose).**

**Note:** If the color of the sample is orange or violet, add 10  $\mu$ 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 membrane is efficient only at pH  $\leq$ 7.5. Buffer QG contains a pH indicator which is yellow at pH  $\leq$ 7.5 and orange or violet at higher pH, allowing easy determination of the optimal pH for DNA binding.

- 6. Add 1 gel volume of isopropanol to the sample and mix by inverting the tube several times.**

For example, if the agarose gel slice is 100 mg, add 100  $\mu$ l isopropanol. Do not centrifuge the sample at this stage.

- 7. To bind DNA, pipet the sample onto the MinElute column and apply vacuum. After the sample has passed through the column, switch off vacuum source.**

For maximum recovery, transfer all traces of sample to the column.

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

- 8. Add 500  $\mu$ l of Buffer QG to the MinElute column and apply vacuum.**
- 9. To wash, add 750  $\mu$ l of Buffer PE to the MinElute 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.

- 10. Transfer the MinElute column to a clean 1.5 ml microfuge tube or to a provided 2 ml collection tube. Centrifuge for 1 min at  $\geq$ 10,000 x g.**

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

- 11. Place the MinElute column in a clean 1.5 ml microcentrifuge tube.**

- 12. To elute DNA, add 10  $\mu$ l of Buffer EB (10 mM Tris-Cl, pH 8.5) or water to the center of the membrane, let the columns stand for 1 min, and then centrifuge for 1 min.**

**IMPORTANT:** Ensure that the elution buffer is dispensed directly onto the center of the membrane for complete elution of bound DNA. The average eluate volume is 9  $\mu$ l from 10  $\mu$ l elution buffer volume.

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  $-20^{\circ}\text{C}$  as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

- 13. If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.**

Loading dye contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange G) that facilitate estimation of DNA migration distance and optimization of agarose gel run time. Refer to Table 3 (page 15) to identify the dyes according to migration distance and agarose gel percentage and type.

# MinElute Reaction Cleanup Kit Protocol

## using a microcentrifuge

This protocol is designed to purify double-stranded DNA fragments from all enzymatic reactions e.g., restriction digestion and labeling resulting in high end-concentrations of DNA (see page 12). Fragments ranging from 70 bp to 4 kb are purified from enzymes, primers, nucleotides, and salts using the MinElute Reaction Cleanup columns in a microcentrifuge. The DNA-binding capacity of the MinElute columns is 5 µg.

### Important points before starting

- The yellow color of Buffer ERC indicates a pH  $\leq 7.5$ .
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifuge steps are carried out at  $\geq 10,000 \times g$  in a conventional tabletop microcentrifuge at room temperature.

### Procedure

1. **Add 300 µl of Buffer ERC to the enzymatic reaction and mix. The maximum volume of enzymatic reaction that can be processed per MinElute column is 100 µl.**

If the enzymatic reaction is in a volume of  $< 20 \mu\text{l}$ , adjust the volume to  $20 \mu\text{l}$ . If the enzymatic reaction exceeds  $100 \mu\text{l}$ , split your reaction, add  $300 \mu\text{l}$  of Buffer ERC to each aliquot of the split reaction, and use the appropriate number of MinElute columns.

2. **Check that the color of the mixture is yellow (similar to Buffer ERC without the enzymatic reaction).**

If the color of the mixture is orange or violet, add  $10 \mu\text{l}$  of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.

3. **Place a MinElute column in a 2 ml collection tube in a suitable rack.**
4. **To bind DNA, apply the sample to the MinElute column and centrifuge for 1 min.**  
To obtain maximal recovery, transfer all traces of sample to the spin column.
5. **Discard the flow-through and place the MinElute column back into the same tube.**
6. **To wash, add 750 µl Buffer PE to the MinElute column and centrifuge for 1 min.**
7. **Discard the flow-through and place the MinElute column back in the same tube. Centrifuge the column for an additional 1 min at maximum speed.**

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

8. **Place the MinElute column in a clean 1.5 ml microcentrifuge tube.**

9. To elute DNA, add 10  $\mu$ l Buffer EB (10 mM Tris-Cl, pH 8.5) or water to the center of the 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 membrane for complete elution of bound DNA. The average volume of eluate is 9  $\mu$ l from 10  $\mu$ 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  $-20^{\circ}\text{C}$  as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

10. If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.

Loading dye contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange G) that facilitate estimation of DNA migration distance and optimization of agarose gel run time. Refer to Table 3 (page 15) to identify the dyes according to migration distance and agarose gel percentage and type.

# MinElute Reaction Cleanup Kit Protocol

## using a vacuum manifold

MinElute columns can also be used on any vacuum manifold with luer connectors (e.g., QIAvac 24 Plus or QIAvac 6S with Luer Adapters). The following protocol is designed to purify double-stranded DNA from all enzymatic reactions e.g., restriction digestion and labeling resulting in high end-concentrations of DNA (see page 12). Fragments ranging from 70 bp to 4 kb are purified from enzymes, primers, nucleotides, and salts using vacuum-driven sample processing.

### Important points before starting

- The yellow color of Buffer ERC indicates a pH  $\leq 7.5$ .
- 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.

### Procedure

- 1. Add 300  $\mu$ l of Buffer ERC to the enzymatic reaction and mix. The maximum volume of the enzymatic reaction is 100  $\mu$ l.**

If the enzymatic reaction is in a volume of  $<20 \mu$ l, adjust the volume to 20  $\mu$ l. If the enzymatic reaction exceeds 100  $\mu$ l, split the reaction, add 300  $\mu$ l of Buffer ERC to each aliquot of the split reaction, and use the appropriate number of MinElute columns.

- 2. Check that the color of the mixture is yellow (similar to Buffer ERC without the enzymatic reaction).**

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

- 3. Prepare the vacuum manifold and MinElute columns according to step 3a, 3b, or 3c.**

- 3a. QIAvac 24 Plus (see page 34):**

Insert up to 24 MinElute columns into the luer extensions of the QIAvac 24 Plus. Close unused positions with luer caps and connect QIAvac 24 Plus to a vacuum source.

- 3b. QIAvac 6S manifold (see page 35):**

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 MinElute 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.

- 3c. **Other vacuum manifolds:** follow the supplier's instructions. Insert each MinElute column into a luer connector.
4. **To bind DNA, load the samples into the MinElute columns by decanting or pipetting, and apply vacuum. After the samples have passed through the column, switch off the vacuum source.**

For maximum recovery, transfer all traces of sample to the column.

5. **To wash, add 750  $\mu$ l of Buffer PE to each MinElute column and apply vacuum.**
6. **Transfer each MinElute column to a microcentrifuge tube or the provided 2 ml collection tubes. Centrifuge for 1 min at  $\geq 10,000 \times g$ .**

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

7. **Place each MinElute column into a clean 1.5 ml microcentrifuge tube.**
8. **To elute DNA, add 10  $\mu$ l of Buffer EB (10 mM Tris-Cl, pH 8.5) or water to the center of each membrane, let the columns stand for 1 min, and then centrifuge.**

**IMPORTANT:** Ensure that the elution buffer is dispensed directly onto the center of the membrane for complete elution of bound DNA. The average eluate volume is 9  $\mu$ l from 10  $\mu$ l elution buffer volume.

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  $-20^{\circ}\text{C}$  as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

9. **If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.**

Loading dye contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange G) that facilitate estimation of DNA migration distance and optimization of agarose gel run time. Refer to Table 3 (page 15) to identify the dyes according to migration distance and agarose gel percentage and type.

# Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: [www.qiagen.com/FAQ/FAQList.aspx](http://www.qiagen.com/FAQ/FAQList.aspx). The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information or protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit [www.qiagen.com](http://www.qiagen.com)).

## Comments and suggestions

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### 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 buffer (e.g., Buffer EB: 10 mM Tris-Cl, pH 8.5) or water. See "Elution in low-salt solutions", page 14.   |
| c) | Elution buffer incorrectly dispensed   | Add elution buffer to the center of the MinElute membrane to ensure that the buffer completely covers the membrane.   |
| d) | Binding mixture turns orange or violet | The pH in the sample exceeds the buffer capacity of Buffer PB, QG, or ERC respectively. Add 20 $\mu$ 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 samples with slight color changes (orange color), add 10 $\mu$ l sodium acetate. |

### Gel

- |    |  |   |
|----|--|---|
| e) | 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.   |
| f) | 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 $\mu$ 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 $\mu$ l sodium acetate. |

**Gel:** refers to MinElute Gel Extraction Kits only; **PCR:** refers to MinElute PCR Purification Kits only; **Reaction Cleanup:** refers to MinElute Reaction Cleanup Kits only; Other notes refer to all kits.

## Comments and suggestions

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- g) 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.

### **Reaction Cleanup**

- h) Sample volume too high or low  
The sample volume must be in the range of 20–100  $\mu$ l.

### **PCR**

- i) Insufficient/no PCR product  
Estimate DNA recovery by running 10% of PCR product before and after purification on an agarose gel.

### **DNA does not perform well (e.g., in ligation reaction)**

- a) Salt concentration in eluate too high  
Modify the wash step by incubating the MinElute column for 5 min at room temperature after adding 750  $\mu$ l of Buffer PE, then centrifuge.
- b) Eluate contains residual ethanol  
Ensure that the wash flow-through is drained from the collection tube and that the MinElute column is then centrifuged at  $\geq 10,000 \times g$  for 1 min.

### **Gel**

- c) Eluate contaminated with agarose  
The gel slice is incompletely solubilized or weighs  $>400$  mg. Be sure to vortex the gel slice in Buffer QG every 2–3 minutes during the solubilization step.

### **PCR**

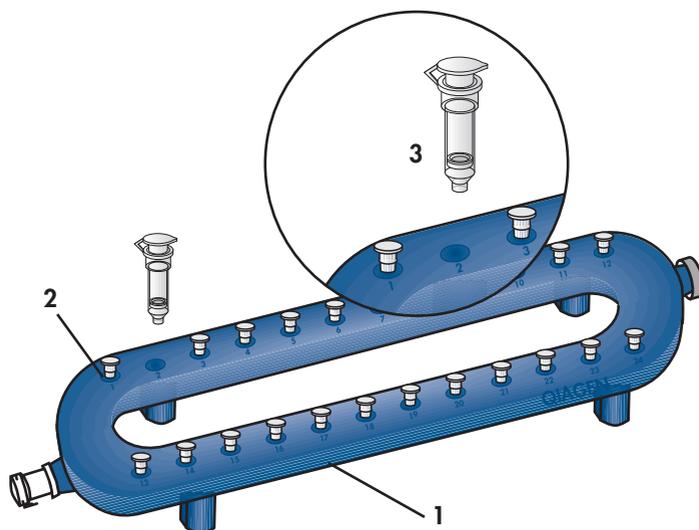
- d) Eluate contains primer-dimers  
Primer-dimers are  $>20$  bp, and are not completely removed. After the binding step, wash the MinElute column with 750  $\mu$ l of a 35% (w/v) guanidine hydrochloride solution (35 g in 100 ml). Continue with the Buffer PE wash step and the elution step as in the protocol.
- e) 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  $95^{\circ}\text{C}$  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.

# Appendix: QIAvac Vacuum Manifolds

## Handling guidelines for QIAvac 24 Plus

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

- Always place the QIAvac 24 Plus on a secure bench top or work area. If dropped, the QIAvac 24 Plus manifold may crack.
- Always store the QIAvac 24 Plus clean and dry. For cleaning procedures see the *QIAvac 24 Plus Handbook*.
- The components of the QIAvac 24 Plus are not resistant to certain solvents (Table 4). 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 Plus 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.



**Figure 6. QIAvac 24 Plus.** Setting up the QIAvac 24 Plus with QIAprep<sup>®</sup>, QIAquick<sup>®</sup>, MinElute, or RNeasy<sup>®</sup> Mini Spin Columns.

1. QIAvac 24 Plus vacuum manifold
2. Luer slot closed with luer plug
3. Spin column\*

\* Not included with the QIAvac 24 Plus. Included in appropriate purification kits.

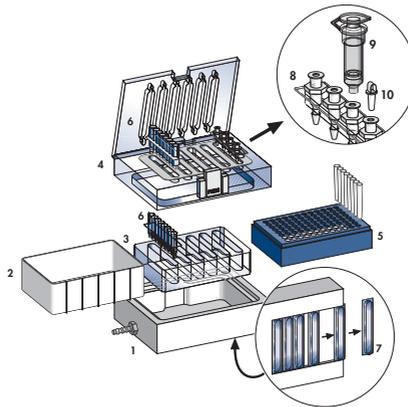
**Table 4. Chemical Resistance Properties of the QIAvac 24 Plus**

<b>Resistant to:</b>		
Acetic acid	Chaotropic salts	Chlorine bleach
Chromic acid	Hydrochloric acid	SDS
Sodium chloride	Sodium hydroxide	Tween 20
Urea		
<b>Not resistant to:</b>		
Benzene	Chloroform	Ethers
Phenol	Toluene	

## Handling guidelines for QIAvac 6S

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

- Always store the 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 5). 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.



**Figure 7. QIAvac 6S.** Components of the QIAvac 6S manifold.

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

\* MinElute kits are not available in 8-well strip format.

<sup>†</sup> Not included with QIAvac 6S. Must be purchased separately.

<sup>‡</sup> Not included with QIAvac 6S. Included in appropriate kits.

**Table 5. Chemical Resistance Properties of the QIAvac 6S**

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

## References

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

# Ordering Information

Product	Contents	Cat. no.
<b>MinElute Kits</b>		
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
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 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 96 UF PCR Purification Kit (4)	4 MinElute 96 UF PCR Purification Plates	28051
MinElute 96 UF PCR Purification Kit (24)	24 MinElute 96 UF PCR Purification Plates	28053
<b>Related products</b>		
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

# Ordering Information

Product	Contents	Cat. no.
<b>Individual Buffers</b>		
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 QG* (250 ml)	250 ml Solubilization and Binding Buffer (with pH indicator)	19063
<b>QIAvac Manifolds and Accessories</b>		
QIAvac 24 Plus	Vacuum manifold for processing 1–24 spin columns: includes QIAvac 24 Plus Vacuum Manifold, Luer Plugs, Quick Couplings	19413
QIAvac 6S	Vacuum manifold for processing 1–24 spin columns: includes Top Plate with flip-up lid, Base, Waste Tray, Blanks, Strip Holder, Rack of Collection Microtubes (1.2 ml)	19503
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 Pump	Universal vacuum pump (capacity 34 L/min, 8 mbar vacuum abs.)	84000‡ 84010§ 84020¶

\* 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.

‡ Japan.

§ North America.

¶ Rest of world.

# Ordering Information

Product	Contents	Cat. no.
<b>QIAcube and accessories</b>		
QIAcube**	Robotic workstation for automated purification of DNA, RNA, or proteins using QIAGEN spin-column kits, 3-year warranty on parts and labor	9001292 <sup>††</sup> 9001293 <sup>†</sup>
Starter Pack, QIAcube <sup>††</sup>	Pack includes: reagent bottle racks (3); rack labeling strips (8); 200 µl filter-tips (1024); 1000 µl filter-tips (1024); 1000 µl filter-tips, wide-bore (1024); 30 ml reagent bottles (18); rotor adapters (120); rotor adapter holder	990395

\*\* Agreements for comprehensive service coverage are available; please inquire.

<sup>††</sup> US, Canada, and Japan.

<sup>††</sup> All starter pack items are available separately.

<sup>†</sup> Rest of world.

**Notes**

## Notes

### Limited License Agreement

Use of this product signifies the agreement of any purchaser or user of the MinElute PCR Purification Kit, the MinElute Gel extraction Kit and the MinElute Reaction Cleanup Kit to the following terms:

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## Bench Protocol: MinElute PCR Purification Microcentrifuge and Vacuum Protocol

New users are strongly advised to familiarize themselves with the detailed protocols and safety information provided in the *MinElute Handbook* before using this bench protocol.

### Notes before starting

- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are carried out at 17,900 x g (13,000 rpm) in a conventional tabletop microcentrifuge at room temperature.
- Add 1:250 volume pH indicator I to Buffer PB. The yellow color of Buffer PB with pH indicator I indicates a pH of  $\leq 7.5$ .

**Note:** If the purified PCR product is to be used in sensitive microarray applications, it may be beneficial to use Buffer PB without addition of pH indicator I. Do not add pH indicator I to buffer aliquots.

### Procedure

1. **Add 5 volumes of Buffer PB to 1 volume of the PCR reaction and mix.**  
If the color of the mixture is orange or violet, add 10  $\mu$ l of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.
2. **Place a MinElute column in ▲ a provided 2 ml collection tube or into ● a vacuum manifold.**  
See the *MinElute Handbook* for details on how to set up a vacuum manifold.
3. **To bind DNA, apply the sample to the MinElute column and ▲ centrifuge for 1 min or ● apply vacuum to the manifold until all samples have passed through the column. ▲ Discard flow-through and place the MinElute column back into the same tube.**
4. **To wash, add 0.75 ml Buffer PE to the MinElute column and ▲ centrifuge for 1 min or ● apply vacuum. ▲ Discard flow-through and place the MinElute column back in the same tube.**
5. **Centrifuge the column in a 2 ml collection tube (provided) for 1 min.**
6. **Place each MinElute column in a clean 1.5 ml microcentrifuge tube.**
7. **To elute DNA, add 10  $\mu$ l of Buffer EB (10 mM Tris-Cl, pH 8.5) or water to the center of the MinElute membrane, let the column stand for 1 min, and then centrifuge the column for 1 min.**
8. **If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.**



## Bench Protocol: MinElute Reaction Cleanup Microcentrifuge and Vacuum Protocol

New users are strongly advised to familiarize themselves with the detailed protocols and safety information provided in *MinElute Handbook* before using this bench protocol.

### Notes before starting

- The yellow color of Buffer ERC indicates a pH  $\leq 7.5$ .
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are carried out at 10,000  $\times g$  in a conventional table-top microcentrifuge.

### Procedure

1. **Add 300  $\mu$ l of Buffer ERC to the enzymatic reaction and mix. Check that the color of the mixture is yellow (similar to Buffer ERC without dissolved agarose).** If the color of the mixture is orange or violet, add 10  $\mu$ l of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.
2. **Place a MinElute column in ▲ a provided 2 ml collection tube or into ● a vacuum manifold.**  
See *MinElute Handbook* for details on how to set up a vacuum manifold.
3. **To bind DNA, apply the sample to the MinElute column and ▲ centrifuge for 1 min or ● apply vacuum to the manifold until all samples have passed through the column. ▲ Discard flow-through and place the MinElute column back into the same tube.**  
The maximum volume of the column reservoir is 800  $\mu$ l. For sample volumes of more than 800  $\mu$ l, simply load and spin again.
4. **To wash, add 0.75 ml of Buffer PE to MinElute column and ▲ centrifuge for 1 min or ● apply vacuum. ▲ Discard flow-through and place the MinElute column back into the same tube.**
5. **Centrifuge the column in a 2 ml collection tube (provided) for 1 min at 10,000  $\times g$ .**
6. **Place MinElute column into a clean 1.5 ml microcentrifuge tube.**
7. **To elute DNA, add 10  $\mu$ l of Buffer EB (10 mM Tris-Cl, pH 8.5) or water to the center of the MinElute membrane, let the column stand for 1 min, and then centrifuge the column for 1 min.**
8. **If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.**



## Bench Protocol: MinElute Gel Extraction Microcentrifuge and Vacuum Protocol

New users are strongly advised to familiarize themselves with the detailed protocols and safety information provided in the *MinElute Handbook* before using this bench protocol.

### Notes before starting

- The yellow color of Buffer QG indicates a pH  $\leq 7.5$ .
- 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 10,000  $\times g$  in a conventional table-top microcentrifuge.

### Procedure

1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.
2. Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer QG to 1 volume of gel (100 mg or approximately 100  $\mu$ l).
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.

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  $\mu$ l of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.

5. Add 1 gel volume of isopropanol to the sample and mix.
6. Place a MinElute spin column in ▲ a provided 2 ml collection tube or into ● a vacuum manifold.

See *MinElute Handbook* for details on how to set up a vacuum manifold.



7. To bind DNA, apply the sample to the MinElute column and ▲ centrifuge for 1 min or ● apply vacuum to the manifold until all samples have passed through the column. ▲ Discard flow-through and place the MinElute column back into the same tube.

The maximum volume of the column reservoir is 800 µl. For sample volumes of more than 800 µl, simply load and spin again.

8. Add 0.5 ml of Buffer QG to the MinElute column and ▲ centrifuge for 1 min or ● apply vacuum. ▲ Discard flow-through and place the MinElute column back into the same tube.
9. To wash, add 0.75 ml of Buffer PE to MinElute column and ▲ centrifuge for 1 min or ● apply vacuum. ▲ Discard flow-through and place the MinElute column back into the same tube.

**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.

10. Centrifuge the column in a 2 ml collection tube (provided) for 1 min at 10,000 x g.
11. Place MinElute column into a clean 1.5 ml microcentrifuge tube.
12. To elute DNA, add 10 µl of Buffer EB (10 mM Tris-Cl, pH 8.5) or water to the center of the MinElute membrane, let the column stand for 1 min, and then centrifuge the column for 1 min.
13. If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.

