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QIAamp® MinElute® Media Handbook

For purification of nucleic acids from liquid media using vacuum systems

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAamp MinElute Media Kit **Catalog no.** **Number of preps**	**(50)** **57414** **50**
QIAamp MinElute Columns (each packaged with a 2 ml Collection Tube)	50
Extension Tubes (3 ml)	50
Collection Tubes (1.5 ml)	50
VacConnectors	50
Buffer ATL	14 ml
Buffer AL*	33 ml
Buffer AW2† (concentrate)	13 ml
Buffer AVE† (tubes with purple caps)	4 x 2 ml
Carrier RNA (tube with red cap)	310 µg
QIAGEN® Proteinase K	1.25 ml
Quick-Start Protocol	1

* Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 4 for safety information.
† Contains sodium azide as a preservative.

Storage

QIAamp MinElute columns should be stored at 2–8°C upon arrival. Short-term storage of up to 4 weeks at room temperature (15–25°C) does not affect performance.

QIAGEN Proteinase K solution can be stored at room temperature for up to one year after delivery, if not otherwise stated on the label. For longer storage or if ambient temperatures exceed 25°C, we suggest storing QIAGEN Proteinase K at 2–8°C.

All buffers can be stored at room temperature.

Lyophilized carrier RNA is stable for up to 1 year when stored at room temperature (15–25°C), if not otherwise stated on the label. Carrier RNA can only be dissolved in Buffer AVE or an internal control (if used); dissolved carrier RNA should be immediately added to Buffer AL as described in "Addition of carrier RNA to Buffer AL" on page 15. This solution should be prepared fresh, and is stable at 2–8°C for up to 48 hours. Unused portions of Buffer AVE-dissolved carrier RNA should be frozen in aliquots at –30°C to –15°C.

Intended Use

The QIAamp MinElute Media Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of disease.

QIAcube® Connect is designed to perform fully automated purification of nucleic acids and proteins in molecular biology applications. The system is intended for use by professional users trained in molecular biological techniques and the operation of QIAcube Connect.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

CAUTION	
	**CAUTION: DO NOT add bleach or acidic solutions directly to waste containing Buffer AL.**

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

Quality Control

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

Introduction

The QIAamp MinElute Media Kit uses well-established QIAamp technology for purification of nucleic acids. The kit combines the selective binding properties of a silica-based membrane with flexible elution volumes of between 20 and 150 µl. The kit is suitable for use with liquid media containing nucleic acids, such as cervical swab transport media (e.g., PreservCyt® or SurePath® solution). Nucleic acids are eluted in Buffer AVE, ready for use in amplification reactions or storage at –30°C to –15°C. Purified nucleic acids are free of proteins, nucleases and other impurities.

Principle and procedure

The QIAamp MinElute Media procedure has 4 steps (lyse, bind, wash, elute; see flowchart, page 8) and uses QIAamp MinElute columns on a vacuum manifold. The procedure is designed to ensure that there is no sample-to-sample cross-contamination and allow safe handling of potentially infectious samples. The simple QIAamp MinElute Media procedure is highly suited for simultaneous processing of multiple samples, and yields pure nucleic acids from 24 samples in less than 90 minutes. The QIAamp MinElute Media procedure can be used for isolation of genomic DNA and viral nucleic acids from a broad range of viruses. However, performance cannot be guaranteed for every virus species and must be validated by the user.

Sample volumes

Each QIAamp MinElute column can bind nucleic acids that are longer than 200 bases, but yield depends on sample volume and nucleic acid content. The procedure is optimized for use with a starting volume of 250 µl.

Lysis with QIAGEN Proteinase K

Samples are lysed under denaturing conditions at elevated temperatures. Lysis is performed in the presence of QIAGEN Proteinase K and Buffer ATL. Addition of Buffer AL enhances lysis efficiency and ensures inactivation of RNases.

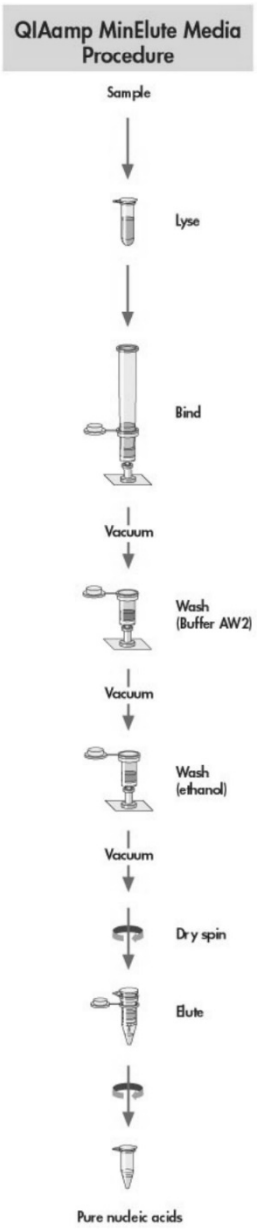
Adsorption to the QIAamp MinElute column membrane

Binding conditions are adjusted by adding ethanol to the lysates to ensure optimal binding of nucleic acids to the QIAamp MinElute column membrane. Lysate is applied to a QIAamp MinElute column and nucleic acids are adsorbed onto the silica membrane as the lysate is drawn through by vacuum pressure. Salt and pH conditions ensure that protein and other contaminants, which can inhibit PCR and other downstream enzymatic reactions, are not retained on the membrane.

A vacuum manifold and a vacuum source or pump capable of producing a vacuum of –800 to –900 mbar are required for the procedure. A vacuum regulator should be used for easy monitoring of vacuum pressures and convenient vacuum release. For details, see “Equipment and Reagents to Be Supplied by User”, page 10.

Removal of residual contaminants

Nucleic acids remain bound to the QIAamp MinElute column membrane, while contaminants are efficiently washed away during a sequence of wash steps using 2 different wash solutions. In a single step, highly pure nucleic acids are eluted in Buffer AVE equilibrated to room temperature (15–25°C).


QIAamp MinElute Media Procedure
Sample
Lyse
Bind
Vacuum
Wash (Buffer AW2)
Vacuum
Wash (ethanol)
Vacuum
Dry spin
Elute
Pure nucleic acids

Automated purification of nucleic acids on QIAcube Instruments

Purification of nucleic acids can be fully automated on QIAcube Connect or the classic QIAcube. The innovative QIAcube instruments use advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using QIAcube instruments follows the same steps as the manual procedure (i.e., lyse, bind, wash and elute), enabling you to continue using the QIAamp MinElute Media Kit for purification of high-quality nucleic acids.

QIAcube instruments are 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/qiacubeprotocols**.

QIAcube Connect.

Equipment and Reagents to Be Supplied by User

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

- Ethanol (96–100%)
- 2 ml microcentrifuge tubes
- Pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- Disposable gloves
  Heating block, thermomixer, or heated orbital incubator for lysis of samples: one for 56°C incubation and another for 70°C incubation
- Microcentrifuge
- Vortexer
  Vacuum manifold (e.g., QIAvac 24 Plus,* cat. no. 19413)
- Vacuum pump with a capacity of 34 liter/min and capable of producing a vacuum of –800 to –900 mbar (e.g., Vacuum Pump,* cat. no. 84000,† 84010,‡ or 84020§)

If using the QIAvac 24 Plus

- QIAvac Connecting System* (cat. no. 19419) — for connection of the QIAvac 24 Plus with the Vacuum Pump (cat. no. 84000,† 84010,‡ or 84020§)

* Available by mid-2004; please check www.qiagen.com/products/accessories
† Japan (110 V, 60 Hz)
‡ Canada and USA (115 V, 60 Hz)
§ Australia and Europe (230 V, 50 Hz)

Important Notes

Elution of pure nucleic acids

DNA is eluted into Buffer AVE. QIAamp MinElute columns allow minimal elution volumes of only 20 µl. Low elution volume leads to highly concentrated nucleic acid eluates.

For downstream applications that require small starting volumes (e.g., some PCR and RT-PCR assays), a more concentrated eluate may increase assay sensitivity.

For downstream applications that require a larger starting volume, elution volume can be increased up to 150 µl. However, an increase in elution volume will decrease the concentration of nucleic acids in the eluate.

The volume of eluate recovered can be up to 5 µl less than the volume of elution buffer applied to the column; for example, an elution buffer volume of 20 µl results in >15 µl final eluate. The volume of eluate recovered also depends on the nature of the sample.

Eluted nucleic acids are collected in 1.5 ml collection tubes (provided). If the purified nucleic acids are to be stored for up to 24 hours, storage at 2–8°C is recommended. To store nucleic acids for longer than 24 hours, storage at –30°C to –15°C is recommended.

Yield and size of nucleic acids

Yields of nucleic acids isolated from liquid media are normally below 1 µg and are therefore difficult to determine with a spectrophotometer. Quantitative amplification methods are recommended for determination of yields. When quantifying nucleic acids isolated using the QIAamp MinElute Media procedure, remember that there will be much more carrier RNA in the sample than target nucleic acids.

The size distribution of nucleic acids purified using this procedure can be checked by agarose gel electrophoresis and hybridization to a target-specific labeled probe followed by autoradiography (Sambrook, J., Fritsch, E.F., Maniatis, T. [1989] *Molecular Cloning: A Laboratory Manual,* 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Carrier RNA

Carrier RNA enhances binding of nucleic acids to the QIAamp MinElute column membrane, especially if there are very few target molecules in the sample. If carrier RNA is not added to Buffer AL, this may lead to reduced nucleic acid recovery.

The amount of lyophilized carrier RNA provided is sufficient for the volume of Buffer AL supplied with the kit. The concentration of carrier RNA has been adjusted so that the QIAamp MinElute Media procedure can be used as a universal purification system compatible with many different amplification systems and is suitable for a wide range of samples.

Different amplification systems vary in efficiency depending on the total amount of nucleic acids present in the reaction. Eluates from this kit contain both target nucleic acids and carrier RNA, and amounts of carrier RNA will greatly exceed amounts of target nucleic acids. The amount of eluate to add to downstream amplifications should therefore be based on the amount of carrier RNA added. To obtain the highest levels of sensitivity in amplification reactions, it may be necessary to adjust the amount of carrier RNA added to Buffer AL.

Addition of internal controls

Using the QIAamp MinElute Media procedure in combination with commercially available amplification systems may require the introduction of an internal control into the procedure. An internal control should be added together with the carrier RNA to Buffer AL. For optimal purification efficiency, internal control molecules should be longer than 200 nucleotides, as smaller molecules are not efficiently recovered.

Refer to the manufacturer's instructions to determine the optimal concentration of internal control. Using a concentration other than that recommended may reduce amplification efficiency.

Handling of QIAamp MinElute columns

Because of the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling QIAamp MinElute columns to avoid cross-contamination between sample preparations:

- Carefully pipet the sample or solution into the QIAamp MinElute column without wetting the rim of the column.
- Avoid touching the QIAamp MinElute column membrane with the pipet tip.
- Change pipet tips between all liquid transfers. The use of aerosol-barrier pipet tips is recommended.
- After all pulse-vortexing steps, briefly centrifuge tubes to remove drops from the inside of the lid.
- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately

Processing QIAamp MinElute columns on the QIAvac 24 Plus

QIAamp MinElute columns are processed on the QIAvac 24 Plus vacuum manifold using disposable VacConnectors and reusable VacValves (use of VacValves is optional). VacValves are inserted directly into the luer extensions/slots of the vacuum manifold, and ensure a steady flow rate, facilitating parallel processing of samples of different nature, volume, or viscosity. If sample flow rates differ significantly, VacValves must be used to ensure consistent vacuum. VacConnectors are disposable connectors that fit between the QIAamp MinElute columns and the VacValves, or between the QIAamp MinElute columns and the luer extensions/slots of the vacuum manifold. VacConnectors prevent direct contact between the QIAamp MinElute

columns and the VacValves or the vacuum manifold during purification, avoiding any cross-contamination between samples. VacConnectors are discarded after a single use.

Handling guidelines for vacuum manifold

If using the QIAvac 24 Plus, refer to the handling guidelines in Appendix A (page 26).

If using another vacuum manifold, refer to the manufacturer's guidelines for handling, cleaning and decontamination.

Centrifugation

For the dry spin at the end of the wash steps of the procedure and for elution, centrifugation should be carried out at full speed.

All centrifugation steps should be carried out at room temperature (15–25°C).

Processing QIAamp MinElute columns in a microcentrifuge

- Close the QIAamp MinElute column before placing it in the microcentrifuge. Centrifuge as described.
- Remove the QIAamp MinElute column and collection tube from the microcentrifuge. Discard the filtrate and the used 2 ml collection tube. Place the QIAamp MinElute column in the 1.5 ml collection tube.
- Open only one QIAamp MinElute column at a time, and take care to avoid generating aerosols.

Preparation of buffers

Addition of carrier RNA to Buffer AL

Add 310 µl Buffer AVE to the tube containing 310 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store at –30°C to –15°C. Do not freeze-thaw the aliquots of carrier RNA more than 3 times.

Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AVE and then added to Buffer AL.

Calculate the volume of Buffer AL/carrier RNA mix needed per batch of samples by selecting the number of samples to be **simultaneously** processed from Table 1. For larger numbers of samples, volumes can be calculated using the following sample calculation:

n x 0.3 ml = **y** ml

y ml x 10 µl/ml = **z** µl

where: **n** = number of samples to be processed simultaneously

y = calculated volume of Buffer AL

z = volume of carrier RNA/Buffer AVE to add to Buffer AL

Gently mix by inverting the tube 10 times. To avoid foaming, do not vortex.

Table 1. Volumes of Buffer AL and Carrier RNA/Buffer AVE Mix required for the QIAamp MinElute Media procedure

No. samples	Vol. Buffer AL, ml	Vol. Carrier RNA/AVE, µl
1	0.3	3
2	0.6	6
3	0.9	9
4	1.2	12
5	1.5	15
6	1.8	18
7	2.1	21
8	2.4	24
9	2.7	27
10	3.0	30
11	3.3	33
12	3.6	36
13	3.9	39
14	4.2	42
15	4.5	45
16	4.8	48
17	5.1	51
18	5.4	54
19	5.7	57
20	6.0	60
21	6.3	63
22	6.6	66
23	6.9	69
24	7.2	72

Note: The sample preparation procedure is optimized for 2.5 µg of carrier RNA per sample. If less carrier RNA has been shown to be better for your amplification system, transfer only the required amount of dissolved carrier RNA to the tubes containing Buffer AL. For each microgram of carrier RNA required per preparation, add 4 µl Buffer AVE-dissolved carrier

RNA per milliliter of Buffer AL. (Use of less than 2.5 µg carrier RNA per sample must be validated for each particular sample type and downstream assay.)

Buffer AW2

Add 30 ml of ethanol (96–100%) to a bottle containing 13 ml of Buffer AW2 concentrate, as described on the bottle. Tick the check box on the label to indicate that ethanol has been added. Store reconstituted Buffer AW2 at room temperature (15–25°C). Reconstituted Buffer AW2 is stable for 1 year when stored at room temperature.

Note: Always mix reconstituted Buffer AW2 by shaking before starting the procedure.

Protocol: Purification of Nucleic Acids from Media

This protocol is for isolation of nucleic acids from 250 µl of liquid media, such as cervical swab transport media (e.g., PreservCyt or SurePath solution).

Important points before starting

- All centrifugation steps are carried out at room temperature (15–25°C).
- After applying lysate or wash solution to the QIAamp MinElute column in steps 12, 13 and 14, wait for at least 1 minute before switching on the vacuum pump. After all liquid has been drawn through the column, wait for at least one minute before switching the vacuum pump off and releasing the vacuum.

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- Equilibrate Buffer AVE to room temperature for elution in step 18.
- If a precipitate has formed in Buffer ATL or AL, dissolve by heating to 70°C and gentle agitation.
- Prepare a 56°C heating block, thermomixer, or heated orbital incubator for use in step 4, and a 70°C heating block, thermomixer, or heated orbital incubator for use in step 7
- Ensure that Buffer AW2 has been prepared according to instructions on page 17.
- Add carrier RNA dissolved in Buffer AVE to Buffer AL according to instructions on page 15.

Procedure

1. Pipet 80 µl of Buffer ATL into a 2 ml microcentrifuge tube (not provided).
2. Add 250 µl of sample into the 2 ml microcentrifuge tube.

3. Add 20 µl QIAGEN Proteinase K. Close the lid and mix by pulse vortexing for 10 s.
4. Incubate at 56°C for 30 min.

   Shake the samples to ensure high nucleic acid yields. For optimal results, use a thermomixer at 900 rpm. If using a heating block, vortex the samples occasionally throughout the incubation period.
5. Briefly centrifuge the 2 ml tube to remove drops from the inside of the lid.
6. Add 250 µl of Buffer AL (containing 10 µg/ml of carrier RNA). Close the lid and mix by pulse-vortexing for 10 s.

   To ensure efficient lysis, it is essential that the sample, Buffer ATL, QIAGEN Proteinase K and Buffer AL are mixed thoroughly to yield a homogeneous solution.

   A white precipitate may form when Buffer AL is added to Buffer ATL. The precipitate does not interfere with the procedure and will dissolve during the incubation in step 7.
7. Incubate at 70°C for 15 min.

   Shake the samples to ensure high nucleic acid yields. For optimal results, use a thermomixer at 900 rpm. If using a heating block, vortex the samples occasionally throughout the incubation period.
8. Briefly centrifuge the 2 ml tube to remove drops from the inside of the lid.
9. Add 300 µl of ethanol (96–100%) to the sample. Close the lid and mix thoroughly by pulse-vortexing for 15 s. Incubate the lysate with the ethanol for 5 min at room temperature (15–25°C).

   Note: If ambient temperature exceeds 25°C, ethanol should be cooled on ice before adding to the lysate.
10. Briefly centrifuge the 2 ml tube to remove drops from the inside of the lid.
11. Insert a QIAamp MinElute column into a VacConnector on the vacuum manifold. Insert an extension tube into the open QIAamp MinElute column.

    Note: Keep the 2 ml collection tube for the dry spin in step 16.

12. Carefully pipet all of the lysate from step 10 into the extension tube of the QIAamp MinElute column. Switch on the vacuum pump. After the lysate has been completely drawn through the column, switch off the vacuum pump, and release the pressure to 0 mbar.

    If the lysate from an individual sample has not completely passed through the membrane despite the VacValves of all other QIAamp MinElute columns being closed, place the QIAamp MinElute column into a clean 2 ml collection tube, close the lid, and centrifuge at full speed for 3 min or until the lysate has completely passed through. Additional 2 ml collection tubes can be purchased separately (see ordering information, page **Error! Bookmark not defined.**).

    Note: For fast and convenient release of vacuum pressure, a vacuum regulator should be used (see "Equipment and Reagents to Be Supplied by User", page 10.)

13. Apply 750 µl of Buffer AW2 into the extension tube of the QIAamp MinElute column. Switch on the vacuum pump. After Buffer AW2 has been completely drawn through the QIAamp MinElute column, switch off the vacuum pump, and release the pressure to 0 mbar.

14. Apply 750 µl of ethanol (96–100%) into the extension tube of the QIAamp MinElute column. Switch on the vacuum pump. After the ethanol has been completely drawn through the QIAamp MinElute column, switch off the vacuum pump, and release the pressure to 0 mbar.

15. Remove and discard the extension tube.

    Note: To avoid cross-contamination, ensure that removed extension tubes do not pass over neighboring QIAamp MinElute columns.

16. Close the lid of the QIAamp MinElute column. Remove it from the vacuum manifold and discard the VacConnector. Place the QIAamp MinElute column in the clean 2 ml collection tube saved from step 11, and centrifuge at full speed (20,000 x *g*, 14,000 rpm) for 3 min to dry the membrane completely.

17. Place the QIAamp MinElute column in a clean 1.5 ml collection tube (provided) and discard the 2 ml collection tube with the filtrate. Open the lid of the QIAamp MinElute column, and incubate the column at room temperature for 15 min.

    Note: Alternatively, for faster incubation, heat the opened QIAamp MinElute column at 56°C for 3 min.

18. Apply 120 µl of Buffer AVE to the center of the membrane in the QIAamp MinElute column. Close the lid and incubate at room temperature for 1 min. Centrifuge at full speed (20,000 x *g*, 14,000 rpm) for 1 min.

    Important: Ensure that Buffer AVE is already equilibrated to room temperature. Incubating the QIAamp MinElute column loaded with Buffer AVE for 5 min at room temperature before centrifugation generally increases yield.

    Elution volume is flexible (20–150 µl) and can be adjusted depending on the downstream application. Reduction of elution volume yields more concentrated eluates, which might increase assay sensitivity. Remember that the recovered eluate volume will differ by ~5 µl from the elution buffer volume applied onto the column.

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**. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit **www.qiagen.com**).

		Comments and suggestions
Little or no nucleic acids in the eluate		
a)	Carrier RNA not added to Buffer AL	Reconstitute carrier RNA in Buffer AVE and mix with Buffer AL as described on page 15. Repeat the purification procedure with new samples.
b)	Degraded carrier RNA	Carrier RNA reconstituted in Buffer AVE was not stored at –30°C to –15°C or underwent multiple freeze– thaw cycles. Alternatively, Buffer AL–carrier RNA mixture was stored for more than 48 hours at 2–8°C. Prepare a new tube of carrier RNA dissolved in Buffer AVE and mix with Buffer AL. Repeat the purification procedure with new samples.
c)	Low concentration of target nucleic acid obtained from the samples	Samples were left at room temperature (15–25°C) for too long. Repeat the purification procedure with new samples.
d)	Insufficient sample lysis in Buffer AL	QIAGEN Proteinase K was subjected to elevated temperatures for a prolonged time. Repeat the purification procedure using new samples and fresh QIAGEN Proteinase K.
e)	Buffer AL–carrier RNA mixture mixed insufficiently	Mix Buffer AL with carrier RNA by gently inverting the tube of Buffer AL–carrier RNA at least 10 times.
f)	Low-percentage ethanol used instead of 96–100%	Repeat the purification procedure with new samples and use 96–100% ethanol.
g)	Buffer AW2 prepared incorrectly	Check that Buffer AW2 concentrate was diluted with the correct volume of ethanol. Repeat the purification procedure with new samples.

		Comments and suggestions
h)	Buffer AW2 prepared with 70% ethanol	Check that Buffer AW2 concentrate was diluted with 96–100% ethanol. Repeat the purification procedure with new samples.
RNA or DNA does not perform well in downstream enzymatic reactions		
a)	Little or no nucleic acids in the eluate	See "Little or no nucleic acids in the eluate" (above) for possible reasons. Increase the amount of eluate added to the reaction, if possible.
b)	Too much or too little carrier RNA in the eluate	Determine the maximum amount of carrier RNA suitable for your amplification reaction. Adjust the concentration of carrier RNA added to Buffer AL accordingly (see "Addition of carrier RNA to Buffer AL", page 15).
c)	Reduced sensitivity	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly. The elution volume can be adjusted proportionally.
d)	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly. The elution volume can be adjusted proportionally.	Salt and ethanol components of Buffer AW2 may have separated out after being unused for a long period. Always mix buffers thoroughly before each preparation.
e)	A new combination of reverse transcriptase and Taq DNA polymerase was used	If enzymes are changed, it may be necessary to readjust the amount of carrier RNA added to Buffer AL and the amount of eluate used.
f)	PCR is inhibited	Depending on the sample type, it may be necessary to include a wash step with Buffer AW1 (not included with the kit; see ordering information, page **Error! Bookmark not defined.**) in the QIAamp MinElute Media procedure. After completing step 12 of the procedure, apply 600 µl ethanol-reconstituted Buffer AW1 into the extension tube of the QIAamp MinElute column. Switch on the vacuum pump. After Buffer AW1 has been completely drawn through the QIAamp MinElute column, switch off the vacuum pump, and release the pressure to 0 mbar. Continue with step 13 of the procedure.

		Comments and suggestions
General handling		
a)	Clogged QIAamp MinElute column	Remove the QIAamp MinElute column from the vacuum manifold, place it in a 2 ml collection tube, and centrifuge at full speed until the sample has completely passed through the membrane.
b)	Variable elution volumes	If different types of sample are processed at the same time on the vacuum manifold, elution volumes may vary between samples.
c)	Vacuum pressure of 800–900 mbar not reached	The VacValves have worn out. Remove all VacValves and insert VacConnectors directly into the luer extensions/slots. Insert QIAamp MinElute columns into the VacConnectors, close the lids of the columns, and switch on the vacuum. Check if vacuum pressure is reached. Replace the VacValves if necessary. Connection to the vacuum pump is leaky. Close all luer slots by attaching luer plugs (if using the QIAvac 24 Plus), and switch on the vacuum pump. Check if vacuum pressure is stable after the pump is switched on (and the vacuum regulator valve is closed). Exchange the connections between pump and vacuum manifold if necessary, ensuring that screw cap (valve) and the quick-coupling female connector are connected to the correct holes of the QIAvac 24 Plus (for details see the *QIAvac 24 Plus Handbook*). If the problem is not resolved after all above checks have been made, replace the vacuum pump with a stronger one.

Appendix A: Handling Guidelines for the QIAvac 24 Plus

The QIAvac 24 Plus, QIAvac Connecting System and Vacuum Pump (see ordering information, page **Error! Bookmark not defined.**) form a complete system for the vacuum processing of QIAamp MinElute columns. The QIAvac 24 Plus is a vacuum manifold which is connected to the Vacuum Pump via the QIAvac Connecting System, which includes tubing, waste bottle, inline filter and vacuum regulator. A valve between the vacuum manifold and the first waste bottle allows control of vacuum in the vacuum manifold.

Up to 24 QIAamp MinElute columns can be connected to the surface of the vacuum manifold. Upon application of vacuum, liquid is drawn through the membranes of the QIAamp MinElute columns into the vacuum manifold, and then collected in the waste bottles.

The following guidelines should be followed when working with the QIAvac 24 Plus vacuum manifold

- Always place the QIAvac 24 Plus on a secure bench top or work area. If dropped, the QIAvac 24 Plus may crack.
- Always store the QIAvac 24 Plus clean and dry.
- To ensure consistent performance, do not apply silicone or vacuum grease to any part of the QIAvac 24 Plus.
- Always use caution and wear safety glasses when working near a manifold under pressure.
- Contact QIAGEN Technical Services or your local distributor for information concerning spare or replacement parts.

- The vacuum pressure is the pressure differential between the inside of the manifold and the atmosphere (standard atmospheric pressure is 1013 millibar or 760 mm Hg) and can be measured using the vacuum regulator. The procedure requires a vacuum pump with a capacity of 34 liter/min and capable of producing a vacuum of –800 to –900 mbar. Higher vacuum pressures must be avoided. Use of vacuum pressures lower than recommended may reduce nucleic acid yield and purity, and increase the frequency of clogged membranes.

Note: If using the VacValves supplied with the QIAamp Vac Accessory Set, VacConnectors must first be inserted into the luer slots of the QIAvac 24 Plus before the VacValves can be inserted.

Setting up the QIAvac 24 Plus

For processing QIAamp MinElute columns on the QIAvac 24 Plus using VacConnectors and VacValves, set up the manifold as follows.

1. Connect the QIAvac 24 Plus to a vacuum source, placing a vacuum trap between the manifold and the source, and a vacuum regulator between the trap and the source. Alternatively, use the QIAvac Connecting System to connect the QIAvac 24 Plus to a vacuum source..
2. Close unused luer slots by attaching closed VacValves.
3. **Optional**: Insert a VacValve into each luer slot used on the QIAvac 24 Plus.

   If flow rates of samples differ significantly, VacValves should be used to ensure consistent vacuum.
4. Insert a VacConnector into each luer slot or VacValve used.

   Perform this step just before starting the purification procedure to avoid exposure of VacConnectors to potential contaminants in the air.

5. Place a QIAamp MinElute column into each VacConnector on the manifold.

   Remove the QIAamp MinElute columns from the blister pack and attach each column to a VacConnector. The collection tubes can be saved for the dry spin in step 16 of the procedure (page 20).

6. Insert an extension tube into each QIAamp MinElute column.

7. For nucleic acid purification, follow the instructions for the QIAamp MinElute Media procedure (page 18). Discard the VacConnectors appropriately after use.

   Leave the lid of the QIAamp MinElute column open while applying vacuum.

   VacValves can be closed individually when each sample is completely drawn through its column, allowing parallel processing of samples of different volumes or viscosities.

8. After processing of samples, discard the liquid waste in the waste bottle appropriately. Clean and decontaminate the vacuum system.

   Note: Buffer AL used in the QIAamp MinElute Media procedure is not compatible with disinfecting agents containing bleach (see safety information, page 4).

Appendix B: Cleaning and Decontaminating the Vacuum Manifold

The QIAvac 24 Plus vacuum manifold must be decontaminated after QIAamp MinElute columns have been processed. The vacuum manifold must also be decontaminated before removal from the laboratory.

Important points before starting

- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Do not use cleaning materials that contain abrasives.
- If the vacuum manifold is still not clean after following the procedure below, soak the vacuum manifold in warm detergent solution for at least 4 hours. Then repeat the procedure.
- Sample-preparation waste may contain guanidine hydrochloride (for details, see the safety information on page 4). Mixing of this chemical with sodium hypochlorite inside a closed container can cause buildup of gas bubbles. Follow the procedure below to avoid this hazard.
- If liquid containing guanidine salt 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% (w/v) sodium hypochlorite.

Procedure

1. Thoroughly rinse the inside and outside of the vacuum manifold, first using laboratory detergent solution, and then using water.
2. Soak the vacuum manifold in 1% (w/v) sodium hypochlorite for 10 min. Thoroughly rinse the inside and outside of the vacuum manifold using water.
3. Wipe the outside of the vacuum manifold using 70% (v/v) ethanol. Then wipe dry.

4. Thoroughly soak the VacValves in laboratory detergent solution. Rinse thoroughly using water.
5. Soak the VacValves in 1% (w/v) sodium hypochlorite for 10 min. Rinse thoroughly using water.
6. Rinse the VacValves using 70% (v/v) ethanol. Wipe dry or allow to air dry.

Ordering Information

Product	Contents	Cat. no.
QIAamp MinElute Media Kit (50)	For 50 minipreps: 50 QIAamp MinElute Columns, QIAGEN Proteinase K, Carrier RNA, Buffers, Extension Tubes (3 ml), Collection Tubes (1.5 ml)	57414
Accessories		
QIAGEN Proteinase K (2 ml)	2 ml (>600 mAU/ml, solution)	19131
Buffer ATL (200 ml)	200 ml Tissue Lysis Buffer	19076
Buffer AL (216 ml)	216 ml Lysis Buffer AL	19075
Buffer AW1 (concentrate, 242 ml)	242 ml Wash Buffer (1) Concentrate	19081
Buffer AW2 (concentrate, 324 ml)	324 ml Wash Buffer (2) Concentrate	19072
Collection Tubes(2 ml)	1000 Collection Tubes (2 ml)	19201
Extension Tubes (3 ml)	For use with QIAGEN Mini or MinElute Columns on vacuum manifolds; 100 per pack	19587
VacConnectors (500)	500 disposable connectors for use with QIAamp spin columns on luer connectors	19407
Vacuum Regulator	For use with QIAvac manifolds	19530
QIAvac 24 Plus	Vacuum manifold for processing 1–24 spin columns	19413
QIAvac Connecting System	System to connect vacuum manifold with vacuum pump	19419

Product	Contents	Cat. no.
Vacuum Pump (110 V, 50/60 Hz)*	Universal vacuum pump (capacity 34 liter/min, 8 mbar vacuum abs.)	84000
Vacuum Pump (115 V, 60 Hz)†	Universal vacuum pump (capacity 34 liter/min, 8 mbar vacuum abs.)	84010
Vacuum Pump (230 V, 50 Hz)‡	Universal vacuum pump (capacity 34 liter/min, 8 mbar vacuum abs.)	84020
QIAcube Connect — for fully automated nucleic acid extraction with QIAGEN spin-column kits		
QIAcube Connect§	Instrument, connectivity package, 1 year warranty on parts and labor	Inquire
Starter Pack, QIAcube	Filter-tips, 200 µl (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), elution tubes (240), rotor adapter holder	990395
Related products		
QIAamp MinElute Virus Vacuum Kit — for simultaneous purification of viral DNA and RNA from plasma, serum and cell-free body fluids using vacuum processing		
QIAamp MinElute Virus Vacuum Kit (50)	For 50 minipreps: 50 QIAamp MinElute Columns, QIAGEN Protease, Carrier RNA, Buffers, Extension Tubes (3 ml), Collection Tubes (1.5 ml)	*57714*
QIAamp MinElute Virus Spin Kit — for simultaneous purification of viral DNA and RNA from plasma, serum and cell-free body fluids using spin processing		
QIAamp MinElute Virus Spin Kit (50)	For 50 minipreps: 50 QIAamp MinElute Columns, QIAGEN Protease, Carrier RNA, Buffers, Collection Tubes (2 ml)	*57704*

Product	Contents	Cat. no.
QIAamp DNA Micro Kit — for purification of genomic and mitochondrial DNA from small amounts of fresh or frozen blood, tissue, forensic samples and dried blood spots		
QIAamp DNA Micro Kit (50)	For 50 DNA preps: 50 QIAamp Min Elute Columns, QIAGEN Proteinase K, Carrier RNA, Buffers, Collection Tubes (2 ml)	56304
QIAamp DNA Mini Kit — for isolation of genomic, mitochondrial, bacterial, parasite or viral DNA		
QIAamp DNA Mini Kit(50)¶	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Buffers, Collection Tubes (2 ml)	51304

* Japan.

† North America.

‡ Rest of world

§ All QIAcube Connect instruments are provided with a region-specific connectivity package, including tablet and equipment necessary to connect to the local network. Further, QIAGEN offers comprehensive instrument service products, including service agreements, installation, introductory training and preventive subscription. Contact your local sales representative to learn about your options.

¶ Other kit sizes are available; see www.qiagen.com.

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Document Revision History

Date	Changes
January 2020	Updated text, ordering information and intended use for QIAcube Connect.

Notes

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