October 2019

QIAamp® Circulating Nucleic Acid Handbook

For concentration and purification of free-circulating DNA, RNA, miRNA, and viral nucleic acids from human plasma, serum, urine, or other cell-free body fluids

Sample to Insight

QIAGEN

Contents

Kit Contents

QIAamp Circulating Nucleic Acid Kit	**(50)**
Catalog no.	**55114**
Number of preps	**50**
QIAGEN® Mini columns	50
Tube extenders (20 ml)	2 x 25
Collection tubes (2.0 ml)	50
Elution tubes (1.5 ml)	50
VacConnectors	50
Buffer ACL*	220 ml
Buffer ACB* (concentrate)	300 ml
Buffer ACW1* (concentrate)	19 ml
Buffer ACW2† (concentrate)	13 ml
Buffer AVE† (purple caps)	5 x 2 ml
QIAGEN proteinase K	4 x 7 ml
Carrier RNA (red caps)	310 µg

* Contains chaotropic salt. See "Safety Information".

† Contains sodium azide as a preservative.

Storage

QIAamp Mini columns should be stored at 2–8°C upon arrival and are stable for at least one year after delivery, if not otherwise stated on the label. However, short-term storage (up to 4 weeks) at room temperature (15–25°C) does not affect their performance. All buffers can be stored at room temperature for up to one year, if not otherwise stated on the label.

Lyophilized carrier RNA can be stored at room temperature. Carrier RNA can only be dissolved in Buffer AVE; dissolved carrier RNA should be immediately added to Buffer ACL as described on Table 2, page 27. This solution should be prepared fresh, and is stable at 2–8°C for up to 48 h. Unused portions of carrier RNA dissolved in Buffer AVE should be frozen in aliquots at –30 to –15°C.

The QIAamp Circulating Nucleic Acid Kit contains a ready-to-use proteinase K solution, which is dissolved in a specially formulated storage buffer. The proteinase K is stable for up to 1 year after delivery when stored at room temperature, if not otherwise stated on the label. To prolong the lifetime of proteinase K, storage at 2–8°C is recommended.

Intended Use

The QIAamp Circulating Nucleic Acid Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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.

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.

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	
	DO NOT add bleach or acidic solution directly to waste containing Buffer ACL, Buffer ACB, or Buffer ACW1.

Buffer ACL, Buffer ACB, and Buffer ACW1 contain guanidine salts, which can form highly reactive compounds when combined with bleach.

If 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.

Quality Control

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

Introduction

Free-circulating nucleic acids, such as tumor-specific extracellular DNA fragments and mRNAs in the blood or fetal nucleic acids in maternal blood, are present in serum or plasma usually as short fragments, <1000 bp (DNA) or <1000 nt (RNA). In addition, free-circulating miRNAs as small as 20 nt have the potential to provide biomarkers for certain cancers and disease states. The concentration of free-circulating nucleic acids in biological fluids, such as plasma, serum, or urine, is usually low and varies considerably among different individuals. For free-circulating DNA in plasma, the concentration can range from 1 to 100 ng/ml in human samples. In samples obtained from different individuals, a similar sample-to-sample variability can be assumed for the concentration of circulating messenger RNA fragments and miRNA molecules.

The QIAamp Circulating Nucleic Acid Kit enables efficient purification of these circulating nucleic acids from human plasma, serum, or urine. Samples can be either fresh or frozen (provided that they have not been frozen and thawed more than once).

Extension tubes and vacuum processing on the QIAvac 24 Plus enable starting sample volumes of up to 5 ml, and flexible elution volumes between 20 µl and 150 µl allow concentration of nucleic acid species that are present in low amounts in the sample material. The kit can also be used for purification and concentration of viral nucleic acids from large sample volumes.

Free-circulating cell-free DNA, RNA or viral nucleic acids are eluted in Buffer AVE, ready for use in amplification reactions or storage at –30 to –15°C. Purified nucleic acids are free of proteins, nucleases, and other impurities.

Principle and procedure

The QIAamp Circulating Nucleic Acid procedure comprises 4 steps (lyse, bind, wash, elute) and is carried out using QIAamp Mini columns on a vacuum manifold. The robust procedure helps to eliminate sample-to-sample cross-contamination and increases user safety when handling potentially infectious samples.

The simple procedure, which is highly suited for simultaneous processing of multiple samples, provides pure nucleic acids in less than 2 h for 24 samples. If the QIAamp Circulating Nucleic Acid Kit is used for isolation of viral RNA and DNA, the performance cannot be guaranteed for every virus species and must be validated by the user.

Sample volumes

QIAamp Mini columns can bind fragmented nucleic acids that are as short as 20 bases, but yield depends on the sample volume and the concentration of circulating nucleic acids in the sample. The QIAamp Circulating Nucleic Acid procedure has been optimized for large sample volumes of up to 5 ml.

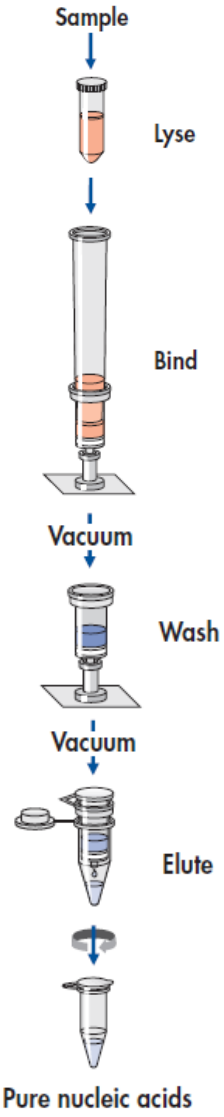


Figure 1. The QIAamp Circulating Nucleic Acid Procedure.

Lysing samples

Free-circulating nucleic acids in biological fluids are usually bound to proteins or enveloped in vesicles, requiring an efficient lysis step to release nucleic acids for selective binding to the QIAamp Mini column. Hence, samples are lysed under highly denaturing conditions at elevated temperatures in the presence of proteinase K and Buffer ACL, which together ensure inactivation of DNases and RNases and complete release of nucleic acids from bound proteins, lipids, and vesicles. For processing of urine samples and for extraction of microRNAs using the microRNA protocol on page 53, Buffer ATL is required as additional lysis buffer to ensure complete release of nucleic acids in the sample. Buffer ATL is available separately (cat. no. 19076; 200 ml).

Adsorption to the QIAamp Mini membrane

Binding conditions are adjusted by adding Buffer ACB to allow optimal binding of the circulating nucleic acids to the silica membrane. Lysates are then transferred onto a QIAamp Mini column, and circulating nucleic acids are adsorbed from a large volume onto the small silica membrane as the lysate is drawn through by vacuum pressure. Salt and pH conditions ensure that proteins and other contaminants, which can inhibit PCR and other downstream enzymatic reactions, are not retained on the QIAamp Mini membrane.

A vacuum manifold (e.g., the QIAvac 24 Plus with the QIAvac Connecting System) and a vacuum pump capable of producing a vacuum of –800 to –900 mbar (e.g., QIAGEN Vacuum Pump) are required for the protocol. A vacuum regulator should be used for easy monitoring of vacuum pressures and convenient vacuum release.

Removal of residual contaminants

Nucleic acids remain bound to the membrane, while contaminants are efficiently washed away during 3 wash steps. In a single step, highly pure circulating nucleic acids are eluted in Buffer AVE, equilibrated to room temperature.

Elution of pure nucleic acids

Elution is performed using Buffer AVE. The elution volume can be as low as 50 µl. If higher nucleic acid concentrations are required, the elution volume can be reduced to as low as 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, the 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 eluate volume recovered can be up to 5 µl less than the volume of elution buffer applied to the column; for example, an elution volume of 20 µl results in >15 µl final eluate. The volume of eluate recovered depends on the nature of the sample.

Eluted nucleic acids are collected in 1.5 ml microcentrifuge tubes (provided). If the purified circulating nucleic acids are to be stored for up to 24 h, storage at 2–8°C is recommended. For periods of storage longer than 24 h, storage at –30 to –15°C is recommended.

Yield and size of nucleic acids

Yields of free-circulating nucleic acids isolated from biological samples are normally below 1 µg and are therefore difficult to determine with a spectrophotometer. In addition, carrier RNA present in the extracted nucleic acids is likely to dominate UV absorbance readings (see page 17). Quantitative amplification methods are recommended for determination of yields. The absolute yield of circulating DNA and RNA obtained from a sample using the QIAamp Circulating Nucleic Acid Kit varies considerably between samples from different individuals and also depends on other factors, e.g., certain disease states.

The QIAamp Circulating Nucleic Acid Kit delivers excellent recovery of fragmented nucleic acids as short as 75 bp. The recovery of microRNAs (as short a 20 nt) is further enhanced when "Protocol: Purification of Circulating microRNA from 1 ml, 2 ml, or 3 ml Serum, Plasma, or Urine" is used.

The size distribution of circulating nucleic acids purified using this procedure can be checked by agarose gel electrophoresis and hybridization to a target-specific labeled probe (Sambrook, J. and Russell, D.W. [2001] *Molecular Cloning: A Laboratory Manual*, 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press).

Description of protocols

Different protocols are provided in this handbook for different volumes of starting material. "Protocol: Purification of Circulating Nucleic Acids from 1 ml, 2 ml, or 3 ml Serum or Plasma" is for processing >3 ml serum or plasma. "Protocol: Purification of Circulating Nucleic Acids from 4 ml or 5 ml Serum or Plasma" is for processing 4–5 ml serum or plasma. "Protocol: Purification of Circulating Nucleic Acids from 1 ml, 2 ml, or 3 ml Urine" is for processing ≤3 ml urine. "Protocol: Purification of Circulating Nucleic Acids from 4 ml Urine" is for processing up to 4 ml urine.

In addition, "Protocol: Purification of Circulating microRNA from 1 ml, 2 ml, or 3 ml Serum, Plasma, or Urine" is designed for purification of circulating miRNA (including total circulating circulating RNA) from 3 ml of plasma, serum or urine.

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 Circulating Nucleic Acid 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**.

Figure 2. 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.

For all protocols

- Pipettes (adjustable)
- Sterile pipette tips (pipette tips with aerosol barriers are recommended to help prevent cross-contamination)
- Water bath or heating block capable of holding 50 ml centrifuge tubes at 60°C
- Heating block or similar at 56°C (capable of holding 2 ml collection tubes)
- Microcentrifuge
- 50 ml centrifuge tubes
- QIAvac 24 Plus vacuum manifold (cat. no. 19413)
- QIAvac Connecting System (cat. no. 19419) or equivalent
- Vacuum Pump (cat. no. 84010 [USA and Canada], 84000 [Japan], or 84020 [rest of the world]) or equivalent pump capable of producing a vacuum of –800 to –900 mbar
- Ethanol (96–100%)*
- Isopropanol (100%)
- Crushed ice
- Some samples may require dilution with phosphate-buffered saline (PBS)
- **Optional**: VacValves (cat. no. 19408)

For isolation of free-circulating nucleic acids from urine or for isolation of microRNA

- Buffer ATL as additional lysis buffer, cat. no. 19076 (up to 1 ml per sample)

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

Important Notes

Preparation of buffers and reagents

Buffer ACB*

Before use, add 200 ml isopropanol (100%) to 300 ml buffer ACB concentrate to obtain 500 ml Buffer ACB. Mix well after adding isopropanol.

Buffer ACW1*

Before use, add 25 ml ethanol (96–100%) to 19 ml buffer ACW1 concentrate to obtain 44 ml Buffer ACW1. Mix well after adding ethanol.

Buffer ACW2†

Before use, add 30 ml ethanol (96–100%) to 13 ml buffer ACW2 concentrate to obtain 43 ml Buffer ACW2. Mix well after adding ethanol.

Adding carrier RNA to Buffer ACL

Carrier RNA serves 2 purposes. First, it enhances binding of nucleic acids to the QIAamp Mini membrane, especially if there are very few target molecules in the sample. Second, the addition of large amounts of carrier RNA reduces the chance of RNA degradation in the rare event that RNase molecules escape denaturation by the chaotropic salts and detergent in Buffer ACL.*

* Contains chaotropic salt. See page 6 for safety information.
† Contains sodium azide as a preservative.

The amount of lyophilized carrier RNA provided is sufficient for the volume of Buffer ACL supplied with the kit. The recommended concentration of carrier RNA has been adjusted so that the QIAamp Circulating Nucleic Acid protocol can be used as a generic purification system compatible with many different amplification systems and is suitable for a wide range of RNA and DNA targets.

Different amplification systems vary in efficiency depending on the total amount of nucleic acid present in the reaction. Eluates from this kit contain both circulating nucleic acids and carrier RNA, and amounts of carrier RNA will greatly exceed amounts of circulating nucleic acids in most cases. Therefore, quantification of isolated circulating nucleic acids by UV-absorbance reading will not be adequate as the results of such measurements are determined by the presence of carrier RNA. Accordingly, calculations of how much eluate to add to downstream amplifications should be based on the amount of carrier RNA added.

To obtain the highest levels of sensitivity in amplification reactions, it may be necessary to reduce the amount of carrier RNA added to Buffer ACL.

For amplification systems involving oligo dT primers (e.g., the QIAGEN miScript System for quantification of microRNAs), no carrier RNA should be added during isolation of free-circulating nucleic acids.

Add 1550 µl Buffer AVE* to the tube containing 310 µg lyophilized carrier RNA to obtain a solution of 0.2 µg/µl. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at –30 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 ACL. It must first be dissolved in Buffer AVE and then added to Buffer ACL.

* Contains sodium azide as a preservative.

Calculate the volume of Buffer ACL–carrier RNA mix needed per batch of samples according to the tables in the protocols. Select the number of samples to be simultaneously processed.

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

Note: The sample-preparation procedure is optimized for a maximum of 1 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 ACL. For each microgram of carrier RNA required per preparation, add 5 µl dissolved carrier RNA to Buffer ACL. (Use of less than 1 µg carrier RNA per sample may be beneficial and must be validated for each particular sample type and downstream assay.)

Removal of DNA from purified circulating nucleic acids

If a downstream application requires the removal of DNA from the sample, we recommend performing a DNase treatment of the final eluate using the RNase-free DNase Set (cat. no. 79254) — see protocol in Appendix B. If a cleanup step is required after DNase treatment, it is recommended that RNeasy® MinElute® Cleanup Kit (cat. no. 74204) be used. Please follow the protocol in Appendix C.

For RT-PCR and real-time RT-PCR with the purified RNA, QIAGEN offers a range of optimized, ready-to-use kits that provide highly specific and sensitive results. Visit **www.qiagen.com** for details.

For analysis and quantification of circulating mRNA by quantitative RT-PCR, we recommend the generation of cDNA using the QuantiTect® Reverse Transcription Kit (cat. no. 205311 for 50 reactions) which includes a gDNA removal step.

The QIAvac 24 Plus

The QIAvac 24 Plus is designed for fast and efficient vacuum processing of up to 24 QIAGEN spin columns in parallel. Samples and wash solutions are drawn through the column membranes by vacuum instead of centrifugation, providing greater speed and reduced hands-on time in purification procedures.

In combination with the QIAvac Connecting System, the QIAvac 24 Plus can be used as a flow-through system. The sample flow-through is collected in a separate waste bottle.

For maintenance of the QIAvac 24 Plus, refer to the handling guidelines in the *QIAvac 24 Plus Handbook*, **www.qiagen.com/HB-0496**

Processing QIAamp Mini columns on the QIAvac 24 Plus

QIAamp Mini columns are processed on the QIAvac 24 Plus using disposable VacConnectors and reusable VacValves. VacValves (optional) are inserted directly into the luer slots of the QIAvac 24 Plus manifold and ensure a steady flow rate, facilitating parallel processing of samples of different natures (e.g., blood and body fluids), volumes or viscosities. They should be used if sample flow rates differ significantly to ensure consistent vacuum. VacConnectors are disposable connectors that fit between QIAamp Mini columns and VacValves or between the QIAamp Mini columns and the luer slots of the QIAvac 24 Plus. They prevent direct contact between the spin column and VacValve during purification, thereby avoiding any cross-contamination between samples. VacConnectors are discarded after a single use. Due to the large solution volumes used, the QIAvac Connecting System (or similar setup with waste bottles) is required (see Figure 3).

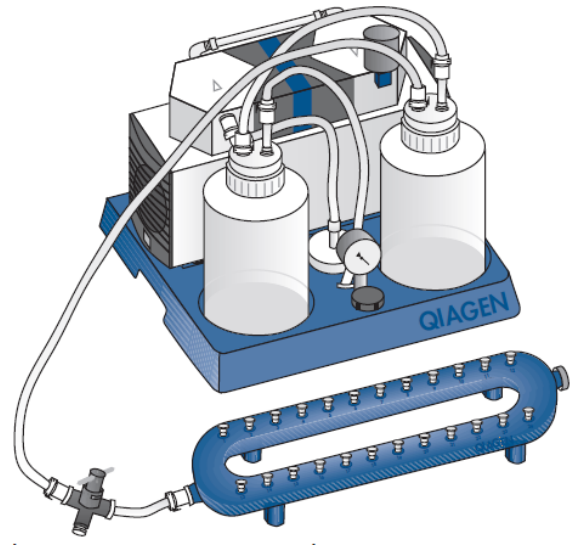


Figure 3. QIAvac 24 Plus, QIAvac Connecting System and Vacuum Pump.

Handling guidelines for the 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 1). If these solvents are spilt 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.

- The vacuum pressure is the pressure differential between the inside of the vacuum manifold and the atmosphere (standard atmospheric pressure 1013 millibar or 760 mm Hg) and can be measured using the QIAvac Connecting System or a vacuum regulator. The protocols require a vacuum pump capable of producing a vacuum of –800 to –900 mbar (e.g., QIAGEN, Vacuum Pump). Higher vacuum pressures must be avoided. Use of vacuum pressures lower than recommended may reduce nucleic acid yield and purity and increase the risk of clogged membranes.

Table 1. Chemical resistance properties of QIAvac 24 Plus

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

1. Connect the QIAvac 24 Plus to a vacuum source. If using the QIAvac Connecting System, connect the system to the manifold and vacuum source as described in Appendix A of the *QIAvac 24 Plus Handbook*.
2. Insert a VacValve (optional) into each luer slot of the QIAvac 24 Plus that is to be used (see Figure 4). Close unused luer slots with luer plugs or close the inserted VacValve.

   VacValves should be used if flow rates of samples differ significantly to ensure consistent vacuum.

3. Insert a VacConnector into each VacValve (see Figure 3).

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

4. Place the QIAamp Mini columns into the VacConnectors on the manifold (see Figure 4).

   Note: Save the collection tube from the blister pack for use in the purification protocol.

5. Insert a tube extender (20 ml) into each QIAamp Mini column (see Figure 4).

   Note: Make sure that the tube extender is firmly inserted into the QIAamp Mini column to avoid leakage of sample.

6. For nucleic acid purification, follow the instructions in the protocols. Discard the VacConnectors appropriately after use.

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

   Switch off the vacuum between steps to ensure that a consistent, even vacuum is applied during processing. For faster vacuum release, a vacuum regulator should be used.

   Note: Each VacValve can be closed individually when the sample is completely drawn through the spin column, allowing parallel processing of samples of different volumes or viscosities.

7. After processing samples, clean the QIAvac 24 Plus (see "Cleaning and Decontaminating the QIAvac 24 Plus" in the *QIAvac 24 Plus Handbook*).

   Note: Buffers ACL, ACB, and ACW1 are not compatible with disinfecting agents containing bleach. See "Safety Information".

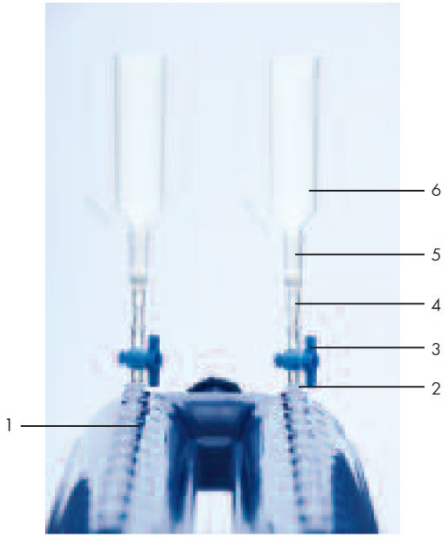


Figure 4. Setting up the QIAvac 24 Plus with QIAamp Mini columns using VacValves, VacConnectors, and Tube Extenders.

1 QIAvac 24 Plus vacuum manifold

2 Luer slot of the QIAvac 24 Plus (closed with luer plug)

3 VacValve*

4 VacConnector

5 QIAamp Mini column

6 Tube Extender

* Must be purchased separately.

We recommend labeling the tubes and the QIAamp Mini columns for use on the QIAvac 24 Plus vacuum system according to the scheme in Figure 6 to avoid the mix-up of samples. This figure can be photocopied and labeled with the names of the samples.

Date: _____

Operator: _____

Run ID: _____

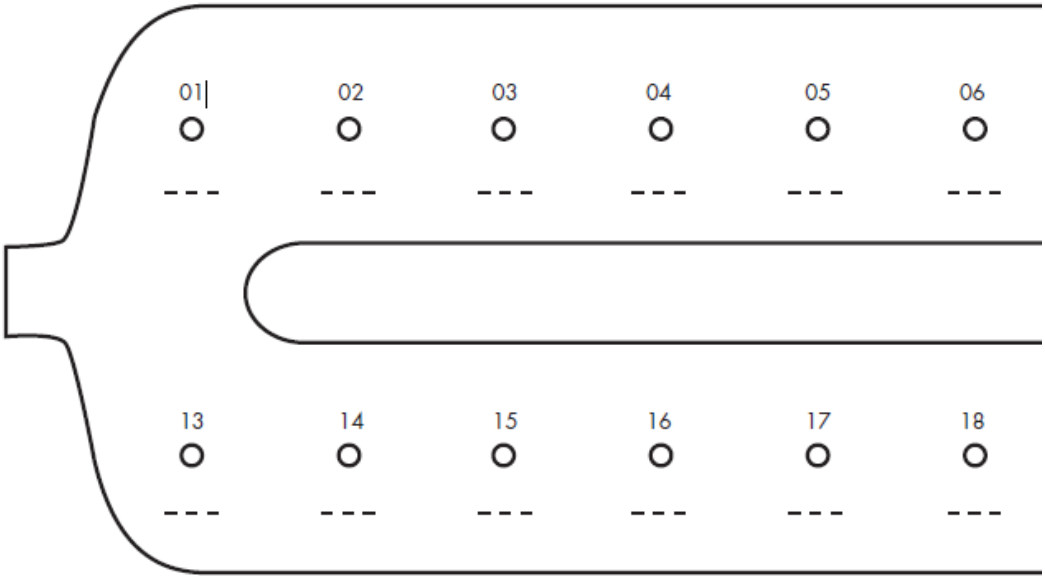
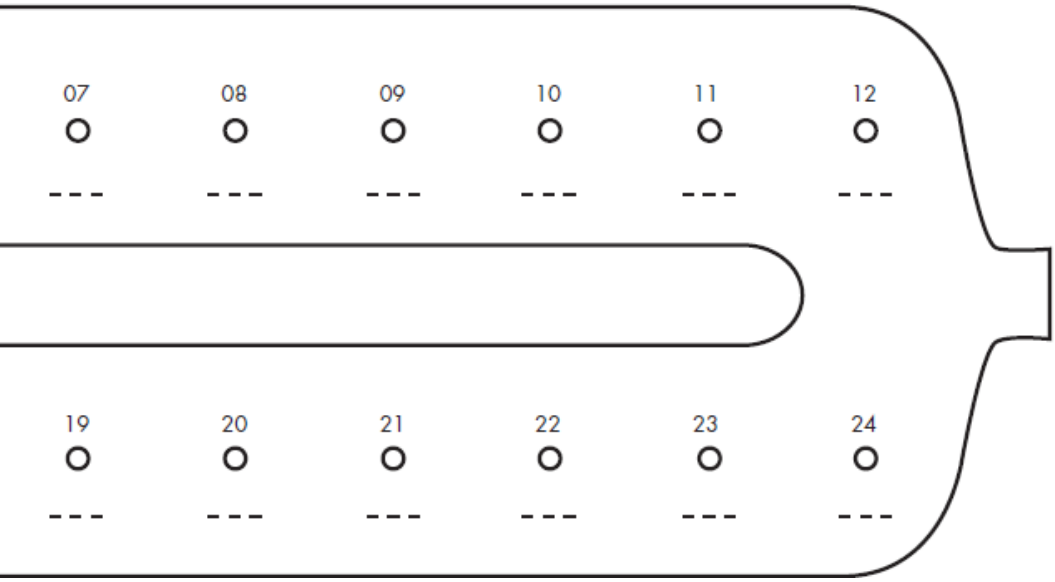


Figure 5. Labeling scheme for tubes and QIAamp Mini columns for use on the QIAvac 24 Plus vacuum system.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Protocol: Purification of Circulating Nucleic Acids from 1 ml, 2 ml, or 3 ml Serum or Plasma

This protocol is for purification of circulating DNA and RNA from 1 ml, 2 ml, or 3 ml of serum or plasma. For 4 ml or 5 ml, see "Protocol: Purification of Circulating Nucleic Acids from 4 ml or 5 ml Serum or Plasma", page 31. For purification of circulating DNA and RNA from urine samples, see "Protocol: Purification of Circulating Nucleic Acids from 1 ml, 2 ml, or 3 ml of Urine", page 36 or "Protocol: Purification of Circulating Nucleic Acids from 4 ml of Urine", page 41. For purification of circulating RNA and miRNA, see "Protocol: Purification of Circulating microRNA from 1 ml, 2 ml, or 3 ml Plasma, Serum, or Urine", page 45.

Important points before starting

- Green (marked with a ■) denotes ■ sample volumes of 1 ml serum or plasma; blue (marked with a ▲) denotes ▲ sample volumes of 2 ml serum or plasma; red (marked with a ●) denotes ● sample volumes of 3 ml serum or plasma.
- All centrifugation steps are carried out at room temperature.
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during protocol steps.

Things to do before starting

- Equilibrate samples to room temperature.
- If samples are ■ <1 ml, ▲ <2 ml, or ● <3 ml, bring the volumes up to ■ 1 ml, ▲ 2 ml, or ● 3 ml with phosphate-buffered saline.
- Set up the QIAvac 24 Plus as described on pages 19–25.
- Heat a water bath or heating block to 60°C for use with 50 ml centrifuge tubes in step 4.
- Heat a heating block to 56°C for use with 2 ml collection tubes in step 14.
- Equilibrate Buffer AVE to room temperature for elution in step 15.

- Ensure that Buffer ACB, Buffer ACW1 and Buffer ACW2 have been prepared according to the instructions in "Important Notes".
- Add carrier RNA reconstituted in Buffer AVE to Buffer ACL according to instructions in Table 2.

Table 2. Volumes of Buffer ACL and carrier RNA7 (dissolved in Buffer AVE) required for processing ■ 1 ml, ▲ 2 ml, or ● 3 ml samples

	Buffer ACL (ml)			
Number of samples	■	▲	●	Carrier RNA in Buffer AVE (µl)
1	0.9	1.8	2.6	5.6
2	1.8	3.5	5.3	11.3
3	2.6	5.3	7.9	16.9
4	3.5	7.0	10.6	22.5
5	4.4	8.8	13.2	28.1
6	5.3	10.6	15.8	33.8
7	6.2	12.3	18.5	39.4
8	7.0	14.1	21.1	45.0
9	7.9	15.8	23.8	50.6
10	8.8	17.6	26.4	56.3
11	9.7	19.4	29.0	61.9
12	10.6	21.1	31.7	67.5
13	11.4	22.9	34.3	73.1
14	12.3	24.6	37.0	78.8
15	13.2	26.4	39.6	84.4
16	14.1	28.2	42.2	90.0
17	15.0	29.9	44.9	95.6
18	15.8	31.7	47.5	101.3
19	16.7	33.4	50.2	106.9
20	17.6	35.2	52.8	112.5
21	18.5	37.0	55.4	118.1
22	19.4	38.7	58.1	123.8
23	20.2	40.5	60.7	129.4
24	21.1	42.2	63.4	135.0

Procedure

1. Pipet ■ 100 µl, ▲ 200 µl or ● 300 µl QIAGEN Proteinase K into a 50 ml centrifuge tube (not provided).
2. Add ■ 1 ml, ▲ 2 ml, or ● 3 ml of serum or plasma to the 50 ml tube.
3. Add ■ 0.8 ml, ▲ 1.6 ml or ● 2.4 ml Buffer ACL (containing 1.0 µg carrier RNA).

   Close the cap and mix by pulse-vortexing for 30 s.

   Make sure that a visible vortex forms in the tube. To ensure efficient lysis, it is essential that the sample and Buffer ACL are mixed thoroughly to yield a homogeneous solution.

   Note: Do not interrupt the procedure at this time. Proceed immediately to step 4 to start the lysis incubation.
4. Incubate at 60°C for 30 min.
5. Place the tube back on the lab bench and unscrew the cap.
6. Add ■ 1.8 ml, ▲ 3.6 ml or ● 5.4 ml Buffer ACB to the lysate in the tube. Close the cap and mix thoroughly by pulse-vortexing for 15–30 s.
7. Incubate the lysate–Buffer ACB mixture in the tube for 5 min on ice.
8. Insert the QIAamp Mini column into the VacConnector on the QIAvac 24 Plus. Insert a 20 ml tube extender into the open QIAamp Mini column.

   Make sure that the tube extender is firmly inserted into the QIAamp Mini column to avoid leakage of sample.

   Note: Keep the collection tube for the dry spin in step 13.

9. Carefully apply the lysate–Buffer ACB mixture from step 7 into the tube extender of the QIAamp Mini column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender.

   Please note that large sample lysate volumes (about 11 ml when starting with 3 ml sample) may need up to 10 min to pass through the QIAamp Mini membrane by vacuum force. For fast and convenient release of the vacuum pressure, the Vacuum Regulator should be used (part of the QIAvac Connecting System).

   Note: To avoid cross-contamination, be careful not to move the tube extenders over neighboring QIAamp Mini Columns.

10. Apply 600 µl Buffer ACW1 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW1 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.

11. Apply 750 µl Buffer ACW2 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW2 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.

12. Apply 750 µl of ethanol (96–100%) to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of ethanol has been drawn through the spin column, switch off the vacuum pump and release the pressure to 0 mbar.

13. Close the lid of the QIAamp Mini column. Remove it from the vacuum manifold, and discard the VacConnector. Place the QIAamp Mini column in a clean 2 ml collection tube, and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.

14. Place the QIAamp Mini Column into a new 2 ml collection tube. Open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.

15. Place the QIAamp Mini column in a clean 1.5 ml elution tube (provided) and discard the 2 ml collection tube from step 14. Carefully apply 20–150 µl of Buffer AVE to the center of the QIAamp Mini membrane. Close the lid and incubate at room temperature for 3 min.

    Important: Ensure that the elution buffer AVE is equilibrated to room temperature. If elution is done in small volumes (<50 µl) the elution buffer has to be dispensed onto the center of the membrane for complete elution of bound DNA.

    Elution volume is flexible and can be adapted according to the requirements of downstream applications. The recovered eluate volume will be up to 5 µl less than the elution volume applied to the QIAamp Mini column.

16. Centrifuge in a microcentrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min to elute the nucleic acids.

Protocol: Purification of Circulating Nucleic Acids from 4 ml or 5 ml Serum or Plasma

This protocol is for purification of circulating DNA and RNA from 4 ml or 5 ml of serum or plasma. For 1 ml, 2 ml, or 3 ml, see "Protocol: Purification of Circulating Nucleic Acids from 1 ml, 2 ml, or 3 ml Serum or Plasma", page 26. For purification of circulating DNA and RNA from urine samples, see "Protocol: Purification of Circulating Nucleic Acids from 1 ml, 2 ml, or 3 ml of Urine", page 36, or "Protocol: Purification of Circulating Nucleic Acids from 4 ml of Urine", page 41. For purification of circulating miRNA, see "Protocol: Purification of Circulating microRNA from 1 ml, 2 ml, or 3 ml Plasma, Serum, or Urine", page 45.

Important points before starting

- Blue (marked with a ▲) denotes ▲ sample volumes of 4 ml serum or plasma; red (marked with a ●) denotes ● sample volumes of 5 ml serum or plasma.
- All centrifugation steps are carried out at room temperature.
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during protocol steps.

Things to do before starting

- Equilibrate samples to room temperature.
- If samples are ▲ >4 ml or ● >5 ml, bring the volumes up to ▲ 4 ml or ● 5 ml with phosphate-buffered saline.
- Set up the QIAvac 24 Plus as described on pages 19–25.
- Heat a water bath or heating block to 60°C for use with 50 ml centrifuge tubes in step 4.
- Heat a heating block to 56°C for use with 2 ml collection tubes in step 14.
- Equilibrate Buffer AVE to room temperature for elution in step 15.

- Ensure that Buffer ACB, Buffer ACW1 and Buffer ACW2 have been prepared according to the instructions in "Important Notes".
- Add carrier RNA reconstituted in Buffer AVE to Buffer ACL according to instructions in Table 3.

Table 3. Volumes of Buffer ACL and carrier RNA (dissolved in Buffer AVE) required for processing ▲ 4 ml or ● 5 ml samples

	Buffer ACL (ml)		
Number of samples	▲	●	Carrier RNA in Buffer AVE (µl)
1	3.5	4.4	5.6
2	7.0	8.8	11.3
3	10.6	13.2	16.9
4	14.1	17.6	22.5
5	17.6	22.0	28.1
6	21.1	26.4	33.8
7	24.6	30.8	39.4
8	28.2	35.2	45.0
9	31.7	39.6	50.6
10	35.2	44.0	56.3
11	38.7	48.4	61.9
12	42.2	52.8	67.5
13	45.8	57.2	73.1
14	49.3	61.6	78.8
15	52.8	66.0	84.4
16	56.3	70.4	90.0
17	59.8	74.8	95.6
18	63.4	79.2	101.3
19	66.9	83.6	106.9
20	70.4	88.0	112.5
21	73.9	92.4	118.1
22	77.4	96.8	123.8
23	81.0	101.2	129.4
24	84.5	105.6	135.0

Procedure

1. Pipet ▲ 400 µl or ● 500 µl QIAGEN Proteinase K into a 50 ml centrifuge tube (not provided).
2. Add ▲ 4 ml or ● 5 ml of serum or plasma to the tube.
3. Add ▲ 3.2 ml or ● 4 ml Buffer ACL (containing 1.0 µg carrier RNA). Close the cap and mix by pulse-vortexing for 30 s.

   Make sure that a visible vortex forms in the tube. To ensure efficient lysis, it is essential that the sample and Buffer ACL are mixed thoroughly to yield a homogeneous solution.

   Note: Do not interrupt the procedure at this time. Proceed immediately to step 4 to start the lysis incubation.
4. Incubate at 60°C for 30 min.
5. Place the tube back on the lab bench and unscrew the cap.
6. Add▲ 7.2 ml or ● 9 ml Buffer ACB to the lysate in the tube. Close the cap and mix thoroughly by pulse-vortexing for 15–30 s.
7. Incubate the lysate–Buffer ACB mixture in the tube for 5 min on ice.
8. Insert the QIAamp Mini column into the VacConnector on the QIAvac 24 Plus. Insert a 20 ml tube extender into the open QIAamp Mini column.

   Make sure that the tube extender is firmly inserted into the QIAamp Mini column to avoid leakage of sample.

   Note: Keep the collection tube for the dry spin in step 13.

9. Carefully apply the lysate–Buffer ACB mixture from step 7 into the tube extender of the QIAamp Mini column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender.

   Please note that large sample lysate volumes (about 20 ml when starting with 5 ml sample) may need up to 15 min to pass through the QIAamp Mini membrane by vacuum force. For fast and convenient release of the vacuum pressure, the Vacuum Regulator should be used (part of the QIAvac Connecting System).

   Note: To avoid cross-contamination, be careful not to move the tube extenders over neighboring QIAamp Mini Columns.

10. Apply 600 µl Buffer ACW1 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW1 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.

11. Apply 750 µl Buffer ACW2 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW2 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.

12. Apply 750 µl of ethanol (96–100%) to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of ethanol has been drawn through the spin column, switch off the vacuum pump and release pressure to 0 mbar.

13. Close the lid of the QIAamp Mini column. Remove it from the vacuum manifold, and discard the VacConnector. Place the QIAamp Mini column in a clean 2 ml collection tube, and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.

14. Place the QIAamp Mini Column into a new 2 ml collection tube. Open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.

15. Place the QIAamp Mini column in a clean 1.5 ml elution tube (provided) and discard the 2 ml collection tube from step 14. Carefully apply 20–150 µl of Buffer AVE to center of the QIAamp Mini membrane. Close the lid and incubate at room temperature for 3 min.

    Important: Ensure that the elution buffer AVE is equilibrated to room temperature. If elution is done in small volumes (<50 µl) the elution buffer has to be dispensed onto the center of the membrane for complete elution of bound DNA.

    Elution volume is flexible and can be adapted according to the requirements of downstream applications. The recovered eluate volume will be up to 5 µl less than the elution volume applied to the QIAamp Mini column.

16. Centrifuge in a microcentrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min to elute the nucleic acids.

Protocol: Purification of Circulating Nucleic Acids from 1 ml, 2 ml, or 3 ml Urine

This protocol is designed for purification of circulating DNA and RNA from up to 1 ml, 2 ml, or 3 ml of urine. For purification of circulating nucleic acids from up to 4 ml of urine, see "Protocol: Purification of Circulating Nucleic Acids from 4 ml of Urine", page 41. For purification of circulating nucleic acids from plasma or serum, see "Protocol: Purification of Circulating Nucleic Acids from 1 ml, 2 ml, or 3 ml Serum or Plasma", page 26, or "Protocol: Purification of Circulating Nucleic Acids from 4 ml or 5 ml Serum or Plasma", page 26. For purification of circulating miRNA, see "Protocol: Purification of Circulating microRNA from 1 ml, 2 ml, or 3 ml Plasma, Serum, or Urine", page 45.

Important points before starting

- Green (marked with a ■) denotes ■ sample volumes of 1 ml urine; blue (marked with a ▲) denotes ▲ sample volumes of 2 ml urine; red (marked with a ●) denotes ● sample volumes of 3 ml urine
- To obtain cell-free nucleic acids from urine, it is recommended to centrifuge urine samples at high speed (e.g., 16,000 x *g*) for 10 min and only use the supernatant for nucleic acid extraction. This will remove cellular material and cellular nucleic acids from the sample.
- All centrifugation steps are carried out at room temperature.
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during protocol steps.

Things to do before starting

- Equilibrate samples to room temperature.
- If samples are ■ <1 ml, ▲ <2 ml, or ● <3 ml, bring the volumes up to ■ 1 ml, ▲ 2 ml, or ● 3 ml with phosphate-buffered saline.
- Set up the QIAvac 24 Plus as described on pages 19–25.
- Heat a water bath or heating block to 60°C for use with 50 ml centrifuge tubes in step 4.
- Heat a heating block to 56°C for use with 2 ml collection tubes in step 14.
- Equilibrate Buffer AVE to room temperature for elution in step 15.
- Ensure that Buffer ACB, Buffer ACW1 and Buffer ACW2 have been prepared according to the instructions in "Important Notes".
- Add carrier RNA reconstituted in Buffer AVE to Buffer ACL according to instructions in Table 4.

Table 4. Volumes of Buffer ACL and carrier RNA (dissolved in Buffer AVE) required for processing■ 1 ml, ▲ 2 ml, or ● 3 ml samples

	Buffer ACL (ml)			
Number of samples	■	▲	●	Carrier RNA in Buffer AVE (µl)
1	0.9	1.8	2.6	5.6
2	1.8	3.5	5.3	11.3
3	2.6	5.3	7.9	16.9
4	3.5	7.0	10.6	22.5
5	4.4	8.8	13.2	28.1
6	5.3	10.6	15.8	33.8
7	6.2	12.3	18.5	39.4
8	7.0	14.1	21.1	45.0
9	7.9	15.8	23.8	50.6
10	8.8	17.6	26.4	56.3
11	9.7	19.4	29.0	61.9
12	10.6	21.1	31.7	67.5
13	11.4	22.9	34.3	73.1
14	12.3	24.6	37.0	78.8
15	13.2	26.4	39.6	84.4
16	14.1	28.2	42.2	90.0
17	15.0	29.9	44.9	95.6
18	15.8	31.7	47.5	101.3
19	16.7	33.4	50.2	106.9
20	17.6	35.2	52.8	112.5
21	18.5	37.0	55.4	118.1
22	19.4	38.7	58.1	123.8
23	20.2	40.5	60.7	129.4
24	21.1	42.2	63.4	135.0

Procedure

1. Pipet ■ 125 µl, ▲ 250 µl, or ● 375 µl QIAGEN Proteinase K into a 50 ml tube (not provided).
2. Add ■ 1 ml, ▲ 2 ml, or ● 3 ml of urine into the 50 ml tube.
3. Add ■ 1 ml, ▲ 2 ml, or ● 3 ml of Buffer ACL (containing 1.0 µg carrier RNA) and ■ 250 µl, ▲ 500 µl, or ● 750 µl Buffer ATL; close the cap and mix by pulse vortexing for 30 s.

   Note: Buffer ATL should be added to the lysis mixture as the last component.

   Make sure a visible vortex forms in the tube. To ensure efficient lysis, it is essential that the sample and Buffers ACL and ATL are mixed thoroughly to yield a homogeneous solution.

   A precipitate may form upon mixing of the components. This will redissolve during the lysis incubation and does not affect the yield of nucleic acids.

   Note: Do not interrupt the procedure at this time. Proceed immediately to step 4 to start the lysis incubation.
4. Incubate at 60°C for 30 min.
5. Place the tube back on the lab bench and unscrew the cap.
6. Add ■ 3.6 ml, ▲ 5.4 ml or ● 7.2 ml of Buffer ACB to the lysate, close the cap, and mix thoroughly by pulse-vortexing for 15–30 s.
7. Incubate the lysate–Buffer ACB mixture for 5 min on ice.
8. Insert the QIAamp Mini column into the VacConnector on the QIAvac 24 Plus. Insert a 20 ml tube extender into the open QIAamp Mini column.

   Make sure that the tube extender is firmly inserted into the QIAamp Mini column To avoid leakage of sample.

   Note: Keep the collection tube for the dry spin in step 13.
9. Carefully apply the lysate from step 7 into the tube extender of the QIAamp Mini column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender.

Note that large sample lysate volumes (about 20 ml when starting with 4 ml urine) may need up to 15 min to pass through the QIAamp Mini membrane by vacuum force. For fast and convenient release of the vacuum pressure, the Vacuum Regulator should be used (part of the QIAvac Connecting System).

Note: To avoid cross contaminations, be careful not to cross neighboring QIAamp columns while tube extenders are removed.

10. Apply 600 µl of Buffer ACW1 to the QIAamp Mini column. Leave the lid of the column open and switch on the vacuum pump. After all of Buffer ACW1 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
11. Apply 750 µl of Buffer ACW2 to the QIAamp Mini column. Leave the lid of the column open and switch on the vacuum pump. After all of Buffer ACW2 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
12. Apply 750 µl of ethanol (96–100%) to the QIAamp Mini column. Leave the lid of the column open and switch on the vacuum pump. After all of the ethanol has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
13. Close the lid of the QIAamp Mini column, remove it from the vacuum manifold and discard the VacConnector. Place the QIAamp Mini column in a clean 2 ml collection tube (saved from step 8) and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.
14. Place the QIAamp Mini column into a new 2 ml collection tube, open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.

    If using the same heating block used in step 4, reduce the temperature by 4°C.
15. Place the QIAamp Mini column in a clean 1.5 ml elution tube and discard the collection tube from step 14. Carefully apply 20–150 µl of Buffer AVE to the center of the QIAamp Mini column membrane. Close the lid and incubate at room temperature for 3 min.
16. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min to elute the nucleic acids.

Protocol: Purification of Circulating Nucleic Acids from 4 ml Urine

This protocol is designed for purification of circulating DNA and RNA from up to 4 ml of urine. For purification of circulating nucleic acids from 1 ml, 2 ml, or 3 ml of urine, see "Protocol: Purification of Circulating Nucleic Acids from 1 ml, 2 ml, or 3 ml of Urine", page 36. For purification of circulating nucleic acids from plasma or serum, see "Protocol: Purification of Circulating Nucleic Acids from 1 ml, 2 ml, or 3 ml Serum or Plasma", page 26 or "Protocol: Purification of Circulating Nucleic Acids from 4 ml or 5 ml Serum or Plasma", page 31. For purification of circulating miRNA, see "Protocol: Purification of Circulating microRNA from 1 ml, 2 ml, or 3 ml Plasma, Serum or Urine", page 45.

Important points before starting

- To obtain cell-free nucleic acids from urine, it is recommended to centrifuge urine samples at high speed (e.g., 16,000 x *g*) for 10 min and only use the supernatant for nucleic acid extraction. This will remove cellular material and cellular nucleic acids from the sample.
- All centrifugation steps are carried out at room temperature.
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during protocol steps.

Things to do before starting

- Equilibrate samples to room temperature.
- Set up the QIAvac 24 Plus as described on pages 19–25.
- Heat a water bath or heating block to 60°C for use with 50 ml centrifuge tubes in step 4.
- Heat a heating block to 56°C for use with 2 ml collection tubes in step 14.
- Equilibrate Buffer AVE to room temperature for elution in step 15.

- Ensure that Buffer ACB, Buffer ACW1 and Buffer ACW2 have been prepared according to the instructions in "Important Notes".
- Add carrier RNA reconstituted in Buffer AVE to Buffer ACL according to instructions in Table 5.

Table 5. Volumes of Buffer ACL and carrier RNA (dissolved in Buffer AVE) required for processing 4 ml samples

Number of samples	Buffer ACL (ml)	Carrier RNA in Buffer AVE (µl)
1	4.4	5.6
2	8.8	11.3
3	13.2	16.9
4	17.6	22.5
5	22.0	28.1
6	26.4	33.8
7	30.8	39.4
8	35.2	45.0
9	39.6	50.6
10	44.0	56.3
11	48.4	61.9
12	52.8	67.5
13	57.2	73.1
14	61.6	78.8
15	66.0	84.4
16	70.4	90.0
17	74.8	95.6
18	79.2	101.3
19	83.6	106.9
20	88.0	112.5
21	92.4	118.1
22	96.8	123.8
23	101.2	129.4
24	105.6	135.0

Procedure

1. Pipet 500 µl QIAGEN Proteinase K into a 50 ml tube (not provided).
2. Add 4 ml of urine into the 50 ml tube.
3. Add 4 ml of Buffer ACL (with carrier RNA as needed) and 1.0 ml Buffer ATL; close the cap and mix by pulse-vortexing for 30 s.

   Note: Buffer ATL should be added to the lysis mixture as the last component. Make sure a visible vortex forms in the tube. To ensure efficient lysis, it is essential that the sample and Buffers ACL and ATL are mixed thoroughly to yield a homogeneous solution.

   A precipitate may form upon mixing of the components. This will redissolve

   during the lysis incubation and does not affect the yield of nucleic acids.

   Note: Do not interrupt the procedure at this time. Proceed immediately to step 4 to start the lysis incubation.
4. Incubate at 60°C for 30 min.
5. Place the tube back on the lab bench and unscrew the cap.
6. Add 9.0 ml of Buffer ACB to the lysate, close the cap, and mix thoroughly by pulse-vortexing for 15–30 s.
7. Incubate the lysate–Buffer ACB mixture for 5 min on ice.
8. Insert the QIAamp Mini column into the VacConnector on the QIAvac 24 Plus.

   Insert a 20 ml tube extender into the open QIAamp Mini column.

   Make sure that the tube extender is firmly inserted into the QIAamp Mini column to avoid leakage of sample.

   Note: Keep the collection tube for the dry spin in step 13.
9. Carefully apply the lysate from step 7 into the tube extender of the QIAamp Mini column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender.

   Please note that large sample lysate volumes (about 20 ml when starting with 4 ml urine) may need up to 15 min to pass through the QIAamp Mini membrane by vacuum force.

For fast and convenient release of the vacuum pressure, the Vacuum Regulator should be used (part of the QIAvac Connecting System).

Note: To avoid cross contaminations, be careful not to cross neighboring QIAamp columns while tube extenders are removed.

10. Apply 600 µl of Buffer ACW1 to the QIAamp Mini column. Leave the lid of the column open and switch on the vacuum pump. After all of Buffer ACW1 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
11. Apply 750 µl of Buffer ACW2 to the QIAamp Mini column. Leave the lid of the column open and switch on the vacuum pump. After all of Buffer ACW2 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
12. Apply 750 µl of ethanol (96–100%) to the QIAamp Mini column. Leave the lid of the column open and switch on the vacuum pump. After all of the ethanol has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
13. Close the lid of the QIAamp Mini column, remove it from the vacuum manifold and discard the VacConnector. Place the QIAamp Mini column in a clean 2 ml collection tube (saved from step 8) and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.
14. Place the QIAamp Mini column into a new 2 ml collection tube, open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.

    If using the same heating block used in step 4, reduce the temperature by 4°C.
15. Place the QIAamp Mini column in a clean 1.5 ml elution tube and discard the collection tube from step 14. Carefully apply 20–150 µl of Buffer AVE to the center of the QIAamp Mini column membrane. Close the lid and incubate at room temperature for 3 min.
16. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min to elute the nucleic acids.

Protocol: Purification of Circulating microRNA from 1 ml, 2 ml, or 3 ml Serum, Plasma, or Urine

This protocol describes how to isolate circulating miRNA (including total circulating RNA) from 1 ml, 2 ml, or 3 ml of plasma, serum or urine. For purification of circulating DNA and RNA from plasma or serum, see "Protocol: Purification of Circulating Nucleic Acids from 1 ml, 2 ml, or 3 ml Serum or Plasma", page 26, or "Protocol: Purification of Circulating Nucleic Acids from 4 ml or 5 ml Serum or Plasma", page 31. For purification of circulating DNA and RNA from urine samples, see "Protocol: Purification of Circulating Nucleic Acids from 1 ml, 2 ml, or 3 ml Urine", page 36, or "Protocol: Purification of Circulating Nucleic Acids from 4 ml of Urine", page 41.

Important points before starting

- Green (marked with a ■) denotes ■ sample volumes of 1 ml samples; blue (marked with a ▲) denotes ▲ sample volumes of 2 ml samples; red (marked with a ●) denotes ● sample volumes of 3 ml samples.
- To obtain cell-free nucleic acids from plasma, serum or urine, it is recommended to centrifuge the sample at high speed (e.g., 16,000 x *g*) for 10 min and only use the supernatant for nucleic acid extraction. This will remove cellular material and cellular nucleic acids from the sample.
- All centrifugation steps are carried out at room temperature.
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during protocol steps.

Things to do before starting

- Equilibrate samples to room temperature.
- If samples are ■ <1 ml, ▲ <2 ml, or ● <3 ml, bring the volumes up to ■ 1 ml, ▲ 2 ml, or ● 3 ml with phosphate-buffered saline.

- Set up the QIAvac 24 Plus as described on pages 19–25.
- Heat a water bath or heating block to 60°C for use with 50 ml centrifuge tubes in step 4.
- Heat a heating block to 56°C for use with 2 ml collection tubes in step 14.
- Equilibrate Buffer AVE to room temperature for elution in step 15.
- Ensure that Buffer ACB, Buffer ACW1 and Buffer ACW2 have been prepared according to the instructions in "Important Notes".
- Add carrier RNA reconstituted in Buffer AVE to Buffer ACL according to instructions in Table 4.

Procedure

1. Pipet ■ 130 µl, ▲ 270 µl, or ● 400 µl QIAGEN Proteinase K into a 50 ml tube (not provided).
2. Add ■ 1 ml, ▲ 2 ml, or ● 3 ml of plasma, serum or urine into the 50 ml tube.
3. Add ■ 1.1 ml, ▲ 2.1 ml, or ● 3.2 ml of Buffer ACL (without carrier RNA) and ■ 0.33 ml, ▲ 0.67 ml, or ● 1.0 ml Buffer ATL; close the cap and mix by pulse-vortexing for 30 s.

   Note: Buffer ATL should be added to the lysis mixture as the last component.

   Make sure a visible vortex forms in the tube. To ensure efficient lysis, it is essential that the sample and Buffers ACL and ATL are mixed thoroughly to yield a homogeneous solution.
4. Incubate at 60°C for 30 min.
5. Place the tube back on the lab bench and unscrew the cap.
6. Add ■ 3.0 ml, ▲ 6.0 ml, or ● 9.0 ml of Buffer ACB and ■ 2.3 ml, ▲ 4.7 ml, or ● 7.0 ml isopropanol to the lysate, close the cap, and mix thoroughly by pulse-vortexing for 15–30 s.
7. Incubate the lysate–Buffer ACB mixture for 5 min on ice.

8. Insert the QIAamp Mini column into the VacConnector on the QIAvac 24 Plus.

   Insert a 20 ml tube extender into the open QIAamp Mini column.

   Make sure that the tube extender is firmly inserted into the QIAamp Mini column to avoid leakage of sample.

   Note: Keep the collection tube for the dry spin in step 13.

9. Carefully apply the lysate from step 7 into the tube extender of the QIAamp Mini column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender.

   Please note that large sample lysate volumes (about 23 ml when starting with 3 ml sample) may need up to 15 min to pass through the QIAamp Mini membrane by vacuum force. For fast and convenient release of the vacuum pressure, the Vacuum Regulator should be used (part of the QIAvac Connecting System).

   Note: To avoid cross contamination, be careful not to cross neighboring QIAamp columns while tube extenders are removed.

10. Apply 600 µl of Buffer ACW1 to the QIAamp Mini column. Leave the lid of the column open and switch on the vacuum pump. After all of Buffer ACW1 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.

11. Apply 750 µl of Buffer ACW2 to the QIAamp Mini column. Leave the lid of the column open and switch on the vacuum pump. After all of Buffer ACW2 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.

12. Apply 750 µl of ethanol (96–100%) to the QIAamp Mini column. Leave the lid of the column open and switch on the vacuum pump. After all of the ethanol has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.

13. Close the lid of the QIAamp Mini column, remove it from the vacuum manifold and discard the VacConnector. Place the QIAamp Mini column in a clean 2 ml collection tube (saved from step 8) and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.
14. Place the QIAamp Mini column into a new 2 ml collection tube, open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.

    If using the same heating block used in step 4, reduce the temperature by 4°C.
15. Place the QIAamp Mini column in a clean 1.5 ml elution tube and discard the collection tube from step 14. Carefully apply 20–150 µl of Buffer AVE to the center of the QIAamp Mini column membrane. Close the lid and incubate at room temperature for 3 min.
16. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 1 min to elute the nucleic acids.

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, visit **www.qiagen.com**).

	Comments and suggestions
Little or no nucleic acids in the eluate	
a) Primary blood tube contains an anticoagulant other than EDTA	Anticoagulants may lead to accelerated DNA degradation compared to other than EDTA blood. Repeat the purification procedure with new samples.
b) Extended time between blood draw and plasma preparation	Blood cells may disintegrate and release genomic DNA into the plasma, diluting the target nucleic acid.
c) Samples frozen and thawed more than once	Repeated freezing and thawing should be avoided as this may lead to DNA degradation. Always use fresh samples or samples thawed only once.
d) Low concentration of target DNA in the samples	Samples were left standing at room temperature for too long. Repeat the purification procedure with new samples.
e) Inefficient sample lysis in Buffer ACL	If QIAGEN Proteinase K was subjected to elevated temperature for a prolonged time, it can lose activity. Repeat the procedure using new samples and fresh QIAGEN Proteinase K.
f) Buffer ACL–carrier RNA mixture not sufficiently mixed	Mix Buffer ACL with carrier RNA by gently inverting the tube of Buffer ACL–carrier RNA at least 10 times.
g) Low-percentage ethanol used instead of 96–100%	Repeat the purification procedure with new samples and 96–100% ethanol. Do not use denatured alcohol, which contains other substances, such as methanol or methylethylketone.
h) Buffer ACB prepared incorrectly	Check that Buffer ACB concentrate was reconstituted with the correct volume of isopropanol (not ethanol, see page 16).
i) Buffer ACW1 or Buffer ACW2 prepared incorrectly	Check that Buffer ACW1 and Buffer ACW2 concentrates were diluted with the correct volume of ethanol (see page 16). Repeat the purification procedure with new samples.

j)	Buffer ACW1 or Buffer ACW2 prepared with 70% ethanol	Check that Buffer ACW1 and Buffer ACW2 concentrates were diluted with 96–100% ethanol (see page 16). Repeat the purification procedure with new samples.

DNA or RNA do not perform well in downstream enzymatic reactions

a)	Little or no DNA 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)	Inappropriate elution volume used	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 adapted proportionally.
c)	Buffers not mixed thoroughly	Salt and ethanol components of wash Buffer ACW2 may have separated out after being left for a long period between runs. Always mix buffers thoroughly before each run.
d)	New Taq DNA polymerase or PCR chemistry	If enzymes are changed, it may be necessary to used readjust the amount of eluate used for PCR.
e)	Interference due to carrier RNA	If the presence of carrier RNA in the eluate interferes with the downstream enzymatic reaction, it may be necessary to reduce the amount of carrier RNA or to omit it altogether.

General handling

a)	Clogged column	QIAamp Mini Close the VacValve, if used, and carefully remove the whole assembly consisting of tube extender, QIAamp Mini column, VacConnector and VacValve from the QIAvac 24 Plus manifold. Carefully transfer the remaining sample lysate from the tube extender to a new 50 ml tube. Remove the QIAamp Mini column from the assembly (see above), place it in a 2 ml collection tube and spin it at full speed for 1 min or until sample has completely passed through the membrane. Re-assemble the QIAamp Mini column with Tube Extender, VacConnector and (optional) VacValve. Transfer the remaining sample lysate into the Tube Extender, switch on the vacuum pump, open the VacValve, and pass the remaining lysate through the QIAamp Mini column. Repeat the above procedure if the QIAamp Mini column continues to clog. Cryoprecipitates may have formed in plasma due to repeated freezing and thawing. These can block the QIAamp Mini column. Do not use plasma that has been frozen and thawed more than once. In case cryoprecipitates are visible clear the sample by centrifugation for 5 min at 16,000 x *g*.
b)	Variable elution volumes	Different samples can affect the volume of the final eluate. The recovered eluate volume will be up to 5 µl less than the elution volume applied to the QIAamp Mini column.

c) Vacuum pressure of 800–900 mbar not reached	The vacuum manifold is not tightly closed. Press down on the lid of the vacuum manifold after the vacuum is switched on. Check if vacuum pressure is reached. Gasket of QIAvac lid has worn out. Check the seal of the manifold visually and replace it if necessary. VacValves have worn out. Remove all VacValves and insert VacConnectors directly into the luer extensions. Insert QIAamp Mini columns into VacConnectors, close the lid of the columns, and switch on vacuum. Check if vacuum pressure is reached. Replace VacValves if necessary. Connection to vacuum pump is leaky. Close all luer extension with luer caps, 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. If the vacuum pressure is still not reached, replace the vacuum pump with a stronger one.

Appendix A: Recommendation for Plasma Separation and Storage

To isolate circulating, cell-free nucleic acids from blood samples, we recommend following this protocol which includes a high g-force centrifugation step to remove cellular debris and thereby reduces the amount of cellular or genomic DNA and RNA in the sample.

1. Place whole EDTA blood in BD Vacutainer® tubes (or other primary blood tubes containing EDTA as anti-coagulant) in centrifuge with swing-out rotor and appropriate buckets.
2. Centrifuge blood samples for 10 min at 1900 x *g* (3000 rpm) and 4°C temperature setting.
3. Carefully aspirate plasma supernatant without disturbing the buffy coat layer.

   About 4–5 ml plasma can be obtained from one 10 ml primary blood tube.

   Note: Plasma can be used for circulating nucleic acid extraction at this stage.

   However, the following high-speed centrifugation will remove additional cellular debris and contamination of the circulating nucleic acids by gDNA and RNA derived from damaged blood cells.
4. Aspirated plasma is transferred into fresh 15 ml centrifuge tubes with conical bottom.
5. Centrifuge plasma samples for 10 min at 16,000 x *g* [in fixed-angle rotor] and 4°C temperature setting.

   This will remove additional cellular nucleic acids attached to cell debris.
6. Carefully remove supernatant to a new tube with a pipette without disturbing the pellet.
7. If plasma will be used for nucleic acid extraction on the same day, store at 2–8°C until further processing. For longer storage, keep plasma frozen at –80°C. Before using the plasma for circulating nucleic acid extraction, thaw plasma tubes at room temperature.

   In case of cryoprecipitates, follow these 2 steps:

   7a. To remove cryoprecipitates, centrifuge plasma sample for 5 min at 16,000 x g (in fixed angle rotor) and 4°C temperature setting.

   7b. Transfer supernatant to new tube and begin with nucleic acid extraction protocol.

Appendix B: DNase Digestion of RNA Before RNA Cleanup

This protocol describes how to digest contaminating DNA in RNA solutions prior to RNA cleanup and concentration. This protocol requires use of the QIAGEN RNase-Free DNase Set (see "Ordering Information"; see the RNase-Free DNase Set product insert for product description and more information).

Important points before starting

- Do not vortex reconstituted DNase I. DNase I is especially sensitive to physical denaturation.
- Mixing should only be carried out by gently inverting the vial.

Things to do before starting

- Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time. Dissolve the lyophilized DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using an RNase-free needle and syringe. Mix gently by inverting the vial. Do not vortex.
- For long-term storage of reconstituted DNase I, remove the stock solution from the glass vial, divide it into single-use aliquots, and store at –20°C for up to 9 months. Thawed aliquots can be stored at 2–8°C for up to 6 weeks. Do not refreeze the aliquots after thawing.

Procedure

1. Mix the following in a microcentrifuge tube:
    - Up to 87.5 µl RNA solution (contaminated with genomic DNA)
    - 10 µl Buffer RDD
    - 2.5 µl DNase I stock solution

    Add RNase-free water until the volume is up to 100 µl. The reaction volumes can be doubled, if necessary, to 200 µl final volume).
2. Incubate on the benchtop (20–25°C) for 10 min.
3. If needed, clean up the RNA according to the protocol described in Appendix C.

Appendix C: Cleanup of RNA Solutions After DNase Treatment (Using the RNeasy MinElute Cleanup Kit)

For "Things to do before starting" and "Safety Information", please refer to the *RNeasy MinElute Cleanup Handbook*, **www.qiagen.com/HB-0486**.

1. Adjust the sample to a volume of 100 µl with RNase-free water. Add 350 µl Buffer RLT, and mix well.
2. Add 700 µl of 96–100% ethanol to the diluted RNA, and mix well by pipetting.

   Do not centrifuge. Proceed immediately to step 3.
3. Transfer 600 µl of the sample to an RNeasy MinElute spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow-through.*

   Transfer the remaining sample (about 550 µl) and repeat the centrifugation. Discard the flow-through.*
4. Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied).

   Add 500 µl Buffer RPE to the spin column. Close the lid gently, and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through. Reuse the collection tube in step 5.

   Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting" in the *RNeasy MinElute Cleanup Handbook*).

* Flow-through contains Buffer RLT and is therefore not compatible with bleach. See "Safety Information" in the *RNeasy MinElute Cleanup Handbook*.

5. Add 500 µl of 80% ethanol to the RNeasy MinElute spin column. Close the lid gently, and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through and collection tube.

   Note: After centrifugation, carefully remove the RNeasy MinElute spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

6. Place the RNeasy MinElute spin column in a new 2 ml collection tube (supplied).

   Open the lid of the spin column, and centrifuge at full speed for 5 min. Discard the flow-through and collection tube.

   To avoid damage to their lids, place the spin columns into the centrifuge with at least one empty position between columns. Orient the lids so that they point in a direction opposite to the rotation of the rotor (e.g., if the rotor rotates clockwise, orient the lids counterclockwise).

   It is important to dry the spin column membrane since residual ethanol may interfere with downstream reactions. Centrifugation with the lids open ensures that no ethanol is carried over during RNA elution.

7. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube (supplied).

   Add 14–20 µl RNase-free water directly to the center of the spin column membrane. Close the lid gently, and centrifuge for 1 min at full speed to elute the RNA.

   As little as 10 µl RNase-free water can be used for elution if a higher RNA concentration is required, but the yield will be reduced by approximately 20%.

   Do not elute with less than 10 µl RNase-free water as the spin column membrane will not be sufficiently hydrated.

   The dead volume of the RNeasy MinElute spin column is 2 µl: elution with 14 µl RNase-free water results in a 12 µl eluate.

Appendix D: General Remarks on Handling RNA

Handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even min amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA To create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and nondisposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water (see "Solutions", page 59). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with a detergent,† thoroughly rinsed, and oven baked at 240°C for at least 4 h (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 h) at 37°C, and then autoclave or heat to 100°C for 15 min to eliminate residual DEPC.

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS),* thoroughly rinsed with RNase-free water, and then rinsed with ethanol‡ and allowed to dry.

* 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.

† 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.

‡ Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 h at 37°C. Autoclave for 15 min to remove any trace of DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris* buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues has been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 min.

* 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.

Ordering Information

Product	Contents	Cat. no.
QIAamp Circulating Nucleic Acid Kit (50)	For 50 preps: QIAamp Mini Columns, Tube Extenders (20 ml), QIAGEN Proteinase K, Carrier RNA, Buffers, VacConnectors and Collection Tubes (1.5 ml and 2 ml)	55114
QIAvac 24 Plus System — for vacuum processing of 1–24 QIAGEN spin columns		
QIAvac 24 Plus	Vacuum Manifold for processing 1–24 spin columns: includes QIAvac 24 Plus Vacuum Manifold, Luer Plugs, Quick Couplings	19413
VacConnectors (500)	500 disposable connectors for use with QIAamp Mini columns on luer slots or VacValves	19407
VacValves (24)	24 valves for use with the QIAvac 24 Plus	19408
Vacuum Regulator	For use with QIAvac manifolds	19530
Vacuum Pump (115 V, 60 Hz) (110 V, 60 Hz)† (230 V, 50 Hz)‡	Universal vacuum pump (capacity 34 L/min, 8 mbar vacuum abs.)	 84010* 84000† 84020‡
QIAvac Connecting System	System to connect vacuum manifold with vacuum pump: includes tray, waste bottles, tubing, couplings, valve, gauge, 24 VacValves	19419

Product	Contents	Cat. no.
RNase-Free DNase Set (50)	1500 units RNase-free DNase I, RNase-free Buffer RDD and RNase-free water for 50 RNA minipreps	79254
QIAGEN Proteinase K (2 ml)	2 ml (>600 mAU/ml, solution)	19131
QIAGEN Proteinase K (10 ml)	10 ml (>600 mAU/ml, solution)	19133
Collection Tubes (2 ml)	1000 Collection Tubes (2 ml)	19201
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	Reagent bottle racks (3), 200 µl filter-tips (1024), 1000 µl filter-tips (1024), 30 ml reagent bottles (12), rotor adapters (240), rotor adapter holder	990395

* US and Canada.

† Japan.

‡ 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.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Date	Changes
10/2019	Updated text and ordering information for QIAcube Connect. Removed “Handbook” from “Kit Contents”.

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