June 2012

QIAamp® UltraSens® Virus Handbook

For highly efficient purification of viral RNA and DNA
from 1 ml plasma and serum samples

QIAGEN®

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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Contents

Kit Contents

QIAamp UltraSens Virus Kit Catalog no. Preps per kit	50 53704 50	250 53706 250
QIAamp Spin Columns	50	250
Collection tubes (2 ml)	150	750
Buffer AC	46 ml	230 ml
Buffer AR*	20 ml	90 ml
Buffer AB (concentrate)	5 ml	22 ml
Buffer AW1* (concentrate)	19 ml	98 ml
Buffer AW2† (concentrate)	13 ml	66 ml
Buffer AVE	3 x 2 ml	10 x 2 ml
Proteinase K	1.25 ml	6 ml
Carrier RNA	310 µg	5 x 310 µg
Selection Guide	1	1

* Contains chaotropic salt which is an irritant. See page 5 for safety information.

† Contains sodium azide. See page 5 for safety information.

Storage

QIAamp spin columns should be stored dry at room temperature (15–25°C); storage at higher temperatures should be avoided. All buffers and reagents should be stored at room temperature unless otherwise stated. After receiving the kit, QIAamp spin columns, buffers, and reagents can be stored for up to 12 months under the above conditions without showing any reduction in performance.

Lyophilized carrier RNA is stable for up to 1 year when stored at room temperature. Carrier RNA dissolved in Buffer AVE (supplied with the kit) should be aliquoted and stored at –20°C (see "Preparation of reagents", page 11). When dissolved in this manner, it is stable for 1 year at –20°C.

The ready-to-use QIAGEN® Proteinase K solution is stable for up to one year after delivery when stored at room temperature. To prolong the lifetime of QIAGEN Proteinase K, storage at 2–8°C is recommended.

Intended Use

The QIAamp UltraSens Virus 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.

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 solutions directly to the sample preparation waste.

Buffers AR and AW1 contain guanidine hydrochloride, 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.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Quality Control

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

Introduction

The QIAamp UltraSens Virus Kit is designed for rapid, highly sensitive, and efficient recovery of viral RNA and DNA from plasma or serum. QIAamp UltraSens technology is used to greatly concentrate viral nucleic acids in samples, allowing downstream detection of very low viral titers. Contaminants and enzyme inhibitors are efficiently removed by the QIAamp purification procedure, which is based on advanced silica-membrane technology and uses no phenol, chloroform, or other organic solvents. The QIAamp UltraSens procedure is designed to allow the inclusion of internal controls with the samples in the initial step, allowing the entire purification process to be accurately monitored. QIAamp purified nucleic acids are highly suited for use in all downstream amplification-based assays.

Principle and procedure

QIAamp UltraSens technology allows viral RNA and DNA from 1 ml plasma or serum samples to be highly concentrated in a single step, prior to purification using QIAamp silica-membrane technology. Ultracentrifugation and specialized laboratory equipment are not required.

Buffer AC contains a reagent that forms complexes with nucleic acids, and these complexes can be sedimented by low–*g*-force centrifugation to form a pellet. This pellet can then be resuspended in a small volume of buffer prior to nucleic acid purification using the QIAamp procedure, enabling extremely efficient and sensitive viral nucleic acid isolation.

Buffer AC and carrier RNA are added to a plasma or serum sample. After a short incubation step, the sample is centrifuged at low speed to pellet the nucleic acid complexes. The supernatant is discarded, and the pellet is resuspended in Buffer AR and proteinase K, and incubated for 10 minutes at 40°C. Binding conditions are adjusted by adding Buffer AB, and the lysate is applied to a QIAamp spin column. During a brief centrifugation, RNA and DNA selectively bind to the QIAamp membrane as contaminants pass through. Remaining contaminants and enzyme inhibitors are efficiently removed by centrifugation in two wash steps, and the pure viral nucleic acids are eluted in low-salt Buffer AVE.

Centrifugation

QIAamp UltraSens technology requires very-low-speed centrifugation. The nucleic acid complexes are sedimented by very low *g*-forces (1200 x *g*). Lower *g*-forces may reduce yield due to incomplete recovery of the complexes, whereas higher *g*-forces can lead to formation of hard pellets that are very difficult to resuspend subsequently. It is also important to centrifuge the QIAamp Spin Column at the recommended speed (3000–5000 x *g*) during the nucleic acid binding step. This ensures maximum nucleic acid recovery.

Note: For best results, depending on your microcentrifuge model, the *g*-force required for centrifugation in step 5 of the protocol may need optimization (see "Important Notes", page 10).

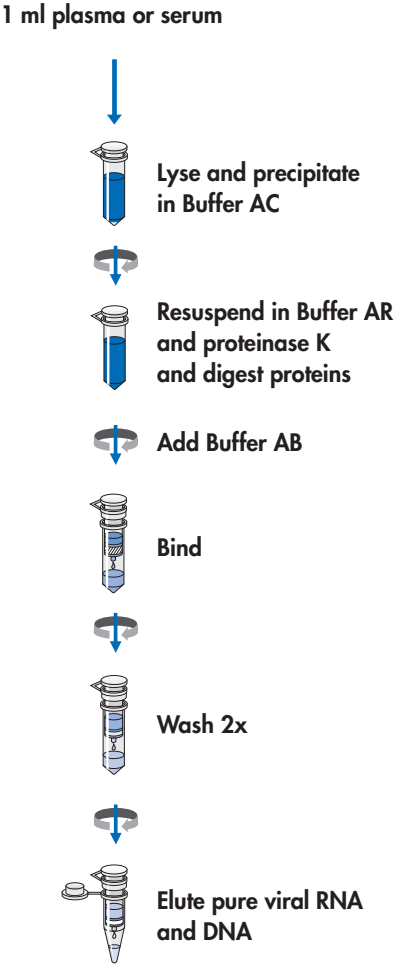
Sample types

The QIAamp UltraSens protocol is designed for isolation of viral nucleic acids (e.g., those of HIV, HCV, or HBV) from 1 ml plasma or serum, or from a 1 ml pool of cell-free body fluids. Such pools can consist of many samples from individual donors, and are usually more homogeneous than samples from single donors.

Note: If you use the QIAamp UltraSens Virus Kit to purify nucleic acids from other types of samples, you may have to optimize the *g*-force used during centrifugation to ensure maximum yield. The optimal *g*-force depends on the type of sample: sample types with lower protein contents than plasma or serum may need a greater *g*-force than the recommended 1200 x *g* to fully pellet the detergent–nucleic acid complexes.

QIAamp UltraSens technology may not work well with plasma samples that contain unusually high amounts of lipids, and recovery of viral nucleic acids from such samples may be reduced. Reduced pellet size can be an indication that the reagent in Buffer AC is interacting with lipids in the sample. See the Troubleshooting Guide on page 17 for strategies to overcome this problem.

The QIAamp UltraSens Virus Procedure



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 %)
- Sterile, RNase-free pipet tips with aerosol barrier
- RNase-free microcentrifuge tubes (1.5 ml and 2 ml)
- Phosphate-buffered saline (PBS) may be required for samples smaller than 1 ml
- Variable-speed microcentrifuge, adjustable between 250 and 1200 x *g*, with a rotor for 2 ml microcentrifuge tubes
- A shaker–incubator, for example the Eppendorf® Thermomixer Compact, or a heating block.

Important Notes

If preparing RNA for the first time, read "Handling RNA" in the Appendix (page 19) before starting. All steps of the protocol should be performed quickly and at room temperature (15–25°C).

Internal controls

An internal control can be added to the sample together with the carrier RNA and Buffer AC in step 2. If only a small volume of internal control is added to the sample, it may be worthwhile to prepare a master mix of carrier RNA and internal control. Thus, a larger volume (which can be more accurately pipetted, reducing sample-to-sample variability) can be added to each sample in only a single pipetting step.

Note: Plasma and serum are rich in RNases. Do not add carrier RNA or an internal control RNA directly to untreated samples.

Optimization of centrifugation

The centrifugation steps (5 and 12) in the protocol are key to optimal nucleic acid recovery. A centrifuge with adjustable *g*-force is highly recommended for use with this protocol. If no such centrifuge is available, the *g*-force can be calculated as follows:

$$rcf = 11.2 \times r \times (rpm/1000)^2$$

Where rcf is the relative centrifugal force (in *g*), r is the radius of the rotor in centimeters, and rpm is the speed of the centrifuge in revolutions per minute.

Step 5: Pelleting the nucleic acid–detergent complexes

Too high a *g*-force can lead to a pellet that is highly compressed and difficult to resuspend. In rare cases, it may be necessary to optimize the *g*-force used in some microcentrifuge models. If the pellet is too solid after centrifugation at 1200 x *g*, decrease the *g*-force in 100 x *g* steps with each preparation to find the *g*-force at which a pellet that is easy to resuspend is formed, but at which the supernatant is free of debris. If debris is present after centrifugation, increase the *g*-force in 100 x *g* steps with each preparation until it is no longer present in the supernatant.

Once the correct *g*-force has been found for a particular centrifuge, it can be used for all plasma and serum samples in that centrifuge — no changes need to be made.

Step 12: Binding nucleic acids to the QIAamp membrane

Centrifugation at 3000–5000 x *g* is necessary at this step to ensure that the complexed nucleic acids have sufficient opportunity to bind to the QIAamp membrane. Higher *g*-forces may lead to loss of nucleic acids in the spin column flow-through.

Sample storage

After collection and centrifugation, plasma or serum samples can be stored at 2–8°C for up to 6 hours. For longer storage, we recommend storing aliquots at –20°C or –80°C. Frozen plasma or serum samples should not be thawed more than once. Repeated freezing and thawing leads to denaturation and precipitation of proteins, causing reduced viral titers and subsequently reduced yields of viral nucleic acids. In addition, cryoprecipitates formed by freeze–thawing accumulate after every cycle, and may interfere with sample preparation, reducing sensitivity. However, in general cryoprecipitates do not need to be removed since they are lysed in Buffer AC at the start of the protocol.

Preparation of reagents

Carrier RNA

Add 310 µl Buffer AVE to each tube of lyophilized carrier RNA to obtain a 1 µg/µl solution. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store at –20°C. Do not freeze–thaw the aliquots of carrier RNA more than three times.

Note: The sample-preparation procedure is optimized for 5.6 µg of carrier RNA per sample. If less carrier RNA has been shown to be better for your particular amplification system, add less carrier RNA to the sample than described in the protocol. (Use of less than 5.6 µg carrier RNA per sample must be validated for each particular sample type.) Discard the unused portion of the reconstituted carrier RNA.

Buffer AB

Before use, add the appropriate amount of ethanol (96–100%) to the Buffer AB concentrate as indicated on the bottle. Buffer AB concentrate is viscous. Mix thoroughly by inverting the bottle ten times to obtain a homogeneous solution. Once the concentrate is dissolved, it is stable at room temperature (15–25°C). Buffer AB dissolved in ethanol is stable for 7 months when stored closed at room temperature.

Buffer AW1*

Add ethanol (96–100%) to Buffer AW1 concentrate before use as indicated on the bottle. Buffer AW1 is stable for 1 year when stored closed at room temperature (15–25°C).

* 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. Not compatible with disinfecting agents that contain bleach, see page 5 for safety information.

Buffer AW2*

Add ethanol (96–100%) to Buffer AW2 concentrate before use as indicated on the bottle. Buffer AW2 is stable for 1 year when stored closed at room temperature (15–25°C).

Handling of QIAamp spin columns

Owing to the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling QIAamp spin columns to avoid cross-contamination:

- Carefully apply the sample to the QIAamp spin column. Pipet the sample into the QIAamp spin column without moistening the rim of the column.
- Change pipet tips between all liquid-transfer steps. The use of aerosol-barrier tips is recommended.
- Avoid touching the QIAamp membrane with the pipet tip.
- After all vortexing steps, briefly centrifuge 1.5 ml microcentrifuge tubes to remove drops from the insides of the lids.
- Wear gloves throughout the procedure. In case of contact between gloves and sample, change the gloves immediately.
- Close the QIAamp spin column before placing it in the microcentrifuge. Centrifuge as described in the protocol. Remove the QIAamp spin column and collection tube from the microcentrifuge. Place the QIAamp spin column in a new collection tube. Discard the filtrate and the collection tube. Please note that the filtrate may contain hazardous waste and should be disposed of properly.
- Open only one QIAamp spin column at a time, and take care to avoid generating aerosols.
- For efficient parallel processing of multiple samples, we recommend filling a rack with collection tubes to which the QIAamp spin columns can be transferred after centrifugation. Used collection tubes containing the filtrate can be discarded, and the new collection tubes containing the QIAamp spin columns placed directly in the microcentrifuge.

* Contains sodium azide. See page 5 for safety information.

Protocol: Purification of Viral RNA and DNA

Important points before starting

- Read "Important Notes" on page 10.
- The use of a shaker–incubator is strongly recommended for use in the incubation steps in the protocol.
- All centrifugation steps should be carried out at room temperature (15–25°C).

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- Check that Buffers AB, AW1, and AW2 and Carrier RNA have been prepared according to the instructions on pages 11 and 12. Mix Buffer AB thoroughly with ethanol by inverting ten times, to ensure that the viscous buffer concentrate is completely dissolved.
- Equilibrate Buffer AR to 60°C in a water bath.

Procedure

1. **Pipet 1 ml plasma or serum equilibrated to room temperature (15–25°C) into a 2 ml microcentrifuge tube (not provided).**

   It is important that plasma is added before Buffer AC.

   If the sample is smaller than 1 ml, adjust to 1 ml using phosphate-buffered saline.

   At least 200 µl of plasma or serum should be processed.

   Ensure that the microcentrifuge tube lid is not contaminated with plasma, since RNases contained in the plasma will immediately degrade the carrier RNA added to the lid in the following step.

2. **Pipet 0.8 ml Buffer AC on top of the sample in the microcentrifuge tube. Pipet 5.6 µl carrier RNA solution into the tube lid.**

   This protocol will also work with smaller amounts of carrier RNA. Using less than 5.6 µg carrier RNA is recommended only when it is known that a higher amount of carrier RNA will interfere with downstream applications. The use of less than 5.6 µg carrier RNA per sample must be validated for each particular sample type.

Note: Do not mix Buffer AC and carrier RNA together before adding them to the sample, as this can lead to variable RNA recovery. After Buffer AC is added to the plasma, RNases are inactivated. An internal control can be added at this step. Pipet the internal control together with the carrier RNA into the microcentrifuge tube lid. If the volume of internal control to be added to the sample is <1 µl, we recommend preparing a master mix of carrier RNA and the internal control, which can then be pipetted into the tube lid. For example, if the master mix contains 0.5 µl internal control per 5.6 µl carrier RNA, pipet 6.1 µl of the mix into the tube lid. As well as saving pipetting steps, this will reduce sample-to-sample variability.

3. **Close the lid and mix thoroughly by first inverting the microcentrifuge tube 3 times and then by vortexing for 10 s.**

   Inverting the tube first is essential because it guarantees that the carrier RNA is completely dissolved in the sample.

4. **Incubate at room temperature for 10 min.**

   A 10 min incubation is sufficient for optimal recovery of HIV, HCV, and HBV. Do not lyse samples for more than 15 min.

5. **Centrifuge the sample at 1200 x *g* for 3 min.**

   This centrifugation step is a key step in the purification procedure. In rare cases, it may be necessary to optimize the centrifugation speed for the microcentrifuge used. If either the pellet is difficult to resuspend after the centrifugation or debris remains in the supernatant, refer to "Optimization of centrifugation" on page 10.

   Following the centrifugation, the supernatant should be clear (with no debris floating around) and the pellet should retain its shape when the microcentrifuge tube is inverted. The pellet may be discolored, but this will not affect the yield or quality of the nucleic acid.

6. **Completely remove and discard the supernatant.**

   To loosen the pellet, it is helpful to do the following: after removing the supernatant, close the tube lid and flick the bottom of the tube several times with your finger while holding the tube by its lid. Vortexing the sample may also help to loosen stubborn pellets. Ensure that no pellet debris remains inside the tube lid.

7. **Add 300 µl Buffer AR warmed to 60°C and 20 µl proteinase K.**

   Preheating Buffer AR to 60°C helps to dissolve the pellet and increases the activity of proteinase K.

   To reduce the number of pipetting steps, a master mix of freshly made warmed Buffer AR and proteinase K can be added to the samples. For example, for 10 samples, mix 3.3 ml of warmed Buffer AR with 220 µl proteinase K (both amounts include 10% excess volume to compensate for pipetting errors). Mix thoroughly by vortexing for 10 s and add 320 µl master mix to each sample.

8. **Vortex thoroughly until the pellet is completely resuspended.**

   Note: It is vital that pellets are completely resuspended to ensure maximum nucleic acid recovery. An efficient way to resuspend multiple samples is to vortex 2 samples simultaneously for 5–10 s and then vortex the next 2 samples, repeating 3–5 more times to completely resuspend each sample. Successively vortexing and then incubating each sample in this way aids the proteinase K digestion.

9. **Incubate for 10 min at 40°C in a mixer–incubator with the mixing speed set to maximum.**

   Note: Ten minutes of digestion with proteinase K at 40°C is sufficient. Do not exceed this incubation time.

   If a mixer–incubator such as an Eppendorf Thermomixer Compact (or similar) is not available, incubate samples on a heating block or in a water bath for 10 min at 40°C, vortexing each sample for 5 s after 5 min and again for 5 s after the 10 min incubation. Do not allow the temperatures of the samples to decrease during vortexing.

10. **Briefly centrifuge to remove drops from the inside of the tube lid.**

11. **Add 300 µl Buffer AB, mix thoroughly by vortexing, and centrifuge briefly to remove drops from the inside of the lid.**

12. **Carefully apply the 700 µl lysate to a QIAamp spin column (sitting in a 2 ml collection tube) without wetting the rim. Close the cap and centrifuge at 3000–5000 x *g* for 1 min.**

    Note: Excessive *g*-forces will lead to inefficient binding of nucleic acids to the silica membrane, resulting in reduced yields.

13. **Place the QIAamp spin column into a new 2 ml collection tube (provided), and discard the tube containing the filtrate. Carefully open the QIAamp spin column and add 500 µl Buffer AW1. Centrifuge at 6000 x *g* for 1 min.**

    Use of a higher *g*-force during centrifugation at this step will not affect the purification process.

14. **Place the QIAamp spin column into a new 2 ml collection tube (provided) and discard the tube containing the filtrate. Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Centrifuge at full speed (20,000 x *g*) for 3 min.**

    Some centrifuge rotors may vibrate upon deceleration, resulting in filtrate contacting the QIAamp spin column. Removing the QIAamp spin column and collection tube from the rotor may also cause the filtrate to come into contact with the bottom of the QIAamp spin column. In these cases, the additional optional step below should be performed.

    - Place the QIAamp spin column in a clean 2 ml collection tube (not provided), and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

15. **Place the QIAamp spin column into a new 1.5 ml microcentrifuge tube (not provided). Discard the collection tube containing the filtrate. Carefully open the QIAamp spin column. To elute viral nucleic acids, carefully apply 30 µl Buffer AVE to the membrane of the spin column. Centrifuge at 6000 x *g* for 1 min.**

    It is important to moisten all of the QIAamp membrane with Buffer AVE to ensure the highest elution efficiency.

16. **Repeat the elution by adding a further 30 µl Buffer AVE and centrifuging at 6000 x *g* for 1 min. A two-step elution ensures maximum recovery of viral nucleic acids.**

    If more eluate is required, increase the amount of Buffer AVE used in the two elution steps (for instance, use 2 x 50 µl instead of 2 x 30 µl) rather than diluting the eluate with Buffer AVE after elution.

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 recovery of nucleic acid	
a) Carrier RNA not added to the sample	Repeat the purification procedure with a new sample, remembering to add carrier RNA.
b) Carrier RNA degraded	Carrier RNA must not contact plasma before Buffer AC is added. Ensure that the inside of the lid is not contaminated with plasma. Add the plasma sample first to the microcentrifuge tube followed by Buffer AC before mixing.
c) Ethanol not added to Buffer AB, AW1, or AW2	Check that ethanol was added to Buffers AB, AW1, and AW2 (see "Preparation of reagents" on page 11). Repeat the purification procedure with a new sample.
d) Inappropriate *g*-force used to centrifuge the nucleic acid complexes or to bind nucleic acids to the silica membrane	Ensure that the *g*-force for each centrifugation step is within the recommended range. Read the comments on page 7 and for protocol steps 5 and 12.
e) High amount of lipid in the sample	If there is a high amount of lipid in a plasma sample, it will appear milky, and the pellet will be small or not present at all. Take a fresh 1.5 ml aliquot of the sample and centrifuge it for 2 min at 10,000 x *g* in a microcentrifuge. The lipid fraction will form a pellet and a thin layer on top of the sample. Pipet 1 ml of the cleared plasma into a new 2 ml microcentrifuge tube (not provided) and process the sample according to the standard protocol.

	Comments and suggestions
e) High amount of lipid in the sample, continued	**Note**: This procedure must be validated for each virus type, e.g., by spiking a known amount of virus into virus-free plasma with a high lipid content and quantifying the amount of viral nucleic acids recovered.
Nucleic acid does not perform well in downstream enzymatic reactions	
a) Reduced sensitivity	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce the volume of eluate added to the amplification reaction if necessary.
b) Buffers AW1 and AW2 used in the wrong order	Ensure that Buffer AW1 and AW2 are used in the correct order in the protocol. Repeat the purification procedure with a new sample.
c) Too much carrier RNA in the eluate	Determine the maximum amount of carrier RNA suitable for your amplification reaction. Adjust the amount of carrier RNA added to the sample accordingly. As little as 1 µg carrier RNA may be sufficient for the purification procedure.
d) Human genomic DNA interfered with the downstream amplification reaction	Human genomic DNA contained in the plasma may be copurified with the viral nucleic acids. For DNA viruses, optimize the PCR conditions to increase specificity. For RNA viruses, optimize RT-PCR conditions or DNase digest the eluate. **Note**: Do not perform a DNase digestion if you wish to detect DNA viruses in the sample. Always use 5.6 µg of carrier RNA if a DNase digestion is performed.

Appendix: General Considerations

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 only minute 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 sample during or after the isolation procedure. To create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non-disposable 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. During the procedure, work quickly to avoid degradation of RNA by endogenous or residual RNases.

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.

Non-disposable plasticware

Non-disposable 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. 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 detergent, thoroughly rinsed and oven baked at >240°C for four or more hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Oven baking will both inactivate ribonucleases and ensure that no other nucleic acids (such as plasmid DNA) are left on the surface of the glassware. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Rinse the glassware with 0.1% DEPC (0.1% in water) at 37°C overnight and then autoclave or heat to 100°C for 15 minutes to remove residual DEPC.

* 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.

Note: Corex® tubes should be rendered RNase-free by treatment with DEPC and not by baking. This will reduce the failure rate of this type of tube during centrifugation.

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS),* rinsed with water, dried with ethanol,† and then filled with a solution of 3% H_2O_2.* After 10 minutes at room temperature (15–25°C), the electrophoresis tanks should be rinsed thoroughly with RNase-free water.

Solutions

Solutions (water and other solutions) should be treated with 0.1% 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. 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. 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 have been modified. Residual DEPC must always be removed from solutions or vessels by autoclaving or heating to 100°C for 15 minutes. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution or let the solution bake for at 37°C 12 hours. Autoclave for 15 minutes to remove any trace of DEPC. It may be desirable to test water sources for the presence of contaminating RNases since many sources of distilled water are free of RNase activity.

Note: QIAamp UltraSens buffers are not rendered RNase-free by DEPC treatment and are therefore free of any DEPC contamination.

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

* 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.

Ordering Information

Product	Contents	Cat. no.
QIAamp UltraSens Virus Kit (50)	For 50 nucleic acid preps: 50 QIAamp Spin Columns, Carrier RNA, Proteinase K, Collection Tubes (2 ml), RNase-free Buffers	53704
QIAamp UltraSens Virus Kit (250)	For 250 nucleic acid preps: 250 QIAamp Spin Columns, Carrier RNA, Proteinase K, Collection Tubes (2 ml), RNase-free Buffers	53706
Related products		
QIAamp MinElute® Virus Kits — for simultaneous purification of viral DNA and RNA from plasma, serum, and cell-free body fluids		
QIAamp MinElute Virus Spin Kit (50)*	For 50 minipreps: 50 QIAamp MinElute Columns, QIAGEN Protease, Carrier RNA, Buffers, Collection Tubes (2 ml)	57704
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 Viral RNA Mini Kit — for isolation of viral RNA from cell-free body fluids		
QIAamp Viral RNA Mini Kit (50)*†	For 50 RNA preps: 50 QIAamp Mini Spin Columns, Carrier RNA, Collection Tubes (2 ml), RNase-free Buffers	52904
QIAcube® — for fully automated sample prep using spin-column kits		
QIAcube	Robotic workstation for automated purification of DNA, RNA, or proteins using QIAGEN spin-column kits, 1-year warranty on parts and labor	Inquire

* Fully automatable on the QIAcube.

† Larger kit sizes available; please inquire.

Ordering Information

Product	Contents	Cat. no.
QuantiTect® Virus Kits — for highly sensitive detection of viral RNA and/or DNA		
QuantiTect Virus Kit (200)*	For 200 x 50 µl reactions: QuantiTect Virus Master Mix (contains ROX™ dye), QuantiTect Virus RT Mix, RNase-Free Water, QuantiTect Nucleic Acid Dilution Buffer	211013
QuantiTect Virus +ROX Vial Kit (200)*	For 200 x 50 µl reactions: QuantiTect Virus NR Master Mix (without ROX dye), ROX Dye Solution, QuantiTect Virus RT Mix, RNase-Free Water, QuantiTect Nucleic Acid Dilution Buffer	211033
QIAGEN OneStep RT-PCR Kit		
QIAGEN OneStep RT-PCR Kit (25)	For 25 reactions: QIAGEN OneStep RT-PCR Enzyme Mix, 5x QIAGEN OneStep RT-PCR Buffer, (containing 12.5 mM $MgCl_2$), dNTP Mix, (containing 5 mM each dNTP), 5x Q-Solution®, RNase-free water	210210
Accessories		
Buffer AW1 (concentrate)	242 ml Wash Buffer 1 Concentrate for 1000 preparations	19081
Buffer AW2 (concentrate)	324 ml Wash Buffer 2 Concentrate for 1000 preparations	19072
Collection Tubes (2 ml)	1000 Collection Tubes (2 ml)	19201
QIAGEN Proteinase K (2)	2 ml (>600 mAU/ml, solution)	19131
QIAGEN Proteinase K (10)	10 ml (>600 mAU/ml, solution)	19133

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* Larger kit sizes available; please inquire.

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