

August 2018

QIAamp[®] DSP Virus Kit Handbook



The QIAamp DSP Virus Kit is a generic system that uses QIAamp technology for isolation and purification of viral nucleic acids from human plasma or serum samples for in vitro diagnostic procedures.

For in vitro diagnostic use

IVD

CE

REF

60704

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Kit Contents

QIAamp DSP Virus Kit			60704
Catalog no.			50
Number of preps			
QIAamp MinElute®	QIAamp MinElute Columns with Wash Tubes (WT) (2 ml)	COL	50
EXT	Column Extenders (3 ml)	COL EXT	50
ET	Elution Tubes (1.5 ml)	ELU TUBE	50
VC	VacConnectors	VAC CON	50
LT	Lysis Tubes (2 ml)	LYS TUBE	50
WT	Wash Tubes (2 ml)	WASH TUBE	50
AL	Lysis Buffer*	LYS BUF	33 ml
AW1	Wash Buffer 1* (concentrate)	WASH BUF 1 CON	19 ml
AW2	Wash Buffer 2† (concentrate)	WASH BUF 2 CON	13 ml
AVE	Elution Buffer† (purple caps)	ELU BUF	4 x 2 ml
PS	Protease Solvent†	QPROT SOLV	4.4 ml
Carrier	Carrier RNA (red caps)	CAR RNA	310
QP	QIAGEN® Protease	QPROT	1 vial

* Contains guanidine hydrochloride. Not compatible with disinfectants containing bleach. See page 8 for safety information.

† Contains sodium azide as a preservative.

‡ Resuspension volume 4.4 ml.

Symbols



Kit contains reagents for 50 sample preparations



Refer to information given in the handbook



To be used by



In vitro diagnostic medical device



Catalog number



Lot number



Material number



Components



Volume



Temperature limitations



Upon arrival



Legal manufacturer



Important note



Change gloves after protocol step with this mark



Open on delivery; store QIAamp Mini Spin Columns at 2–8°C



Global Trade Item Number



Write down the current date after adding ethanol to the bottle



Adding



Contains



Lyophilized



Reconstitute in



Ethanol

GuHCl

MALEIC ACID

SUBT



Guanidine hydrochloride

Maleic acid

Subtilisin

Leads to

Storage

QIAamp MinElute Columns should be stored at 2–8°C upon arrival.

All buffers can be stored at room temperature (15–25°C).

Lyophilized Carrier RNA can be stored at room temperature until the expiration date. Carrier RNA can only be dissolved in Elution Buffer (AVE); dissolved Carrier RNA should be immediately added to Lysis Buffer (AL) as described on page 19. This solution should be prepared fresh and is stable at 2–8°C for up to 48 hours. Unused portions of Carrier RNA dissolved in Elution Buffer (AVE) should be frozen in aliquots at –20°C.

Lyophilized QIAGEN Protease (QP) can be stored at room temperature until the expiration date without decrease in performance.

Reconstituted QIAGEN Protease (QP) is stable for up to 1 year when stored at 2–8°C, but only until the expiration date.

Reconstituted Wash Buffer 1 (AW1) and reconstituted Wash Buffer 2 (AW2) are stable for up to 1 year when stored at room temperature, but only until the expiration date.

Quality Control

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

Intended Use

The QIAamp DSP Virus Kit is a generic system that uses QIAamp technology for isolation and purification of viral nucleic acids from human plasma or serum samples for in vitro diagnostic purposes. Any diagnostic results generated using the sample preparation procedure in conjunction with any downstream diagnostic NAT assay should be interpreted with regard to other clinical or laboratory findings.

The product is intended for use by professional users such as technicians and physicians trained in molecular biological techniques. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modification of DNA or RNA followed by signal detection or amplification. The isolated and purified viral nucleic acids can be used in qualitative (e.g., blood screening) as well as quantitative (e.g., viral load monitoring) diagnostic NAT assays.

To minimize irregularities in diagnostic results, the product is intended to be used with an internal control as well as positive and negative controls throughout the process of sample preparation, and sample amplification and detection according to the downstream assay used.

The product is designed for use with the QIAvac 24 Plus vacuum system or an equivalent vacuum system.

Product Use Limitations

The kit is not for use with blood, tissue, bone marrow or cultured cells. The kit is also not for isolating and purifying bacterial, fungal or parasite nucleic acids. The performance of the kit in isolating and purifying viral nucleic acids from other cell-free body fluids, such as urine and CSF, has not been evaluated.

Warnings and Precautions

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 to the sample preparation waste.

Lysis Buffer (AL) and Wash Buffer 1 (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.

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles to avoid personal injury or injury to others.

QIAGEN has not tested the liquid waste generated by the QIAamp DSP Virus procedure for residual infectious materials. Therefore, universal precautions (gloves, lab coats and eye protection) for handling potentially infectious human source material should be employed while working with this product, and liquid waste must be considered infectious and be handled and discarded according to local safety regulations.

The following hazard and precautionary statements apply to components of the QIAamp DSP Virus Kit.

Buffer AL



Contains: guanidine hydrochloride; maleic acid. Warning! May be harmful if swallowed or if inhaled. Causes skin irritation. May cause an allergic skin reaction. Causes serious eye irritation. Wear protective gloves/protective clothing/eye protection/face protection.

Buffer AW1



Contains: guanidine hydrochloride. Warning! Harmful if swallowed or if inhaled. Causes skin irritation. Causes serious eye irritation. Wear protective gloves/protective clothing/eye protection/face protection.

QIAGEN Protease



Contains: subtilisin. Danger! Harmful if swallowed. Causes skin irritation. Causes serious eye damage. May cause allergy or asthma symptoms or breathing difficulties if inhaled. May cause respiratory irritation. Avoid breathing dust/fume/gas/mist/vapors/spray. Wear protective gloves/protective clothing/eye protection/face protection. Wear respiratory protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF exposed or concerned: Immediately call a POISON CENTER or doctor/physician. Remove victim to fresh air and keep at rest in a position comfortable for breathing.



Introduction

The QIAamp DSP Virus Kit uses well-established technology for simultaneous isolation and purification of viral DNA and RNA. The QIAamp DSP Virus procedure combines the selective binding properties of a silica-based membrane with minimal elution volumes of 20 µl or 60 µl.

The procedure is suitable for use with plasma or serum; either can contain citrate or EDTA. Samples can be either fresh, lyophilized or frozen, provided they have not been frozen and thawed more than once. The procedure can be used for isolation of viral RNA and DNA from a broad range of RNA and DNA viruses. The procedure is designed to avoid sample-to-sample cross-contamination and allow safe handling of potentially infectious samples. The procedure is highly suited for simultaneous processing of multiple samples. Viral nucleic acids are eluted in Elution Buffer (AVE), ready for use in amplification reactions or storage at -20°C .

Principle and procedure

The QIAamp DSP Virus procedure comprises 4 steps:

- Lysing the virus particles in the sample
- Binding the viral nucleic acids in the lysate to the membrane of a QIAamp MinElute Column
- Washing the membrane
- Eluting the viral nucleic acids from the membrane

The procedure is carried out using QIAamp MinElute Columns on a vacuum manifold.

Lysing virus particles

Samples are lysed under denaturing conditions at elevated temperatures. Lysis is performed in the presence of QIAGEN Protease (QP) and Lysis Buffer (AL), which together ensure inactivation of RNases.

Binding nucleic acids to the QIAamp MinElute Column membrane

To optimize the binding of viral DNA and RNA to the QIAamp MinElute Column membrane, ethanol is first added to the lysates. Each lysate is then applied to a QIAamp MinElute Column, and viral nucleic acids are adsorbed onto the silica-based membrane as the lysate is drawn through by vacuum pressure.

Removing residual contaminants

While the viral nucleic acids remain bound to the QIAamp MinElute Column membrane, contaminants are efficiently washed away using first Wash Buffer 1 (AW1), then Wash Buffer 2 (AW2), and then ethanol.

Eluting pure nucleic acids

Viral nucleic acids are eluted from the QIAamp MinElute Column membrane using Elution Buffer (AVE). The QIAamp MinElute Columns allow elution volumes of 20 µl or 60 µl.

Performance characteristics

The linear range of the QIAamp DSP Virus procedure has been determined for HIV RNA and HBV DNA in several downstream diagnostic assays (, and).

Table 1.-Downstream diagnostic assays in which the linear range of the QIAamp DSP Virus Procedure has been tested

Assay	Kit
Real-time RT-PCR of HIV RNA	TaqMan [®] assay and cobas [®] AMPLICOR HIV-1 MONITOR [®] Test
Real-time PCR of HBV DNA	TaqMan assay and cobas AMPLICOR HBV MONITOR [®] Test

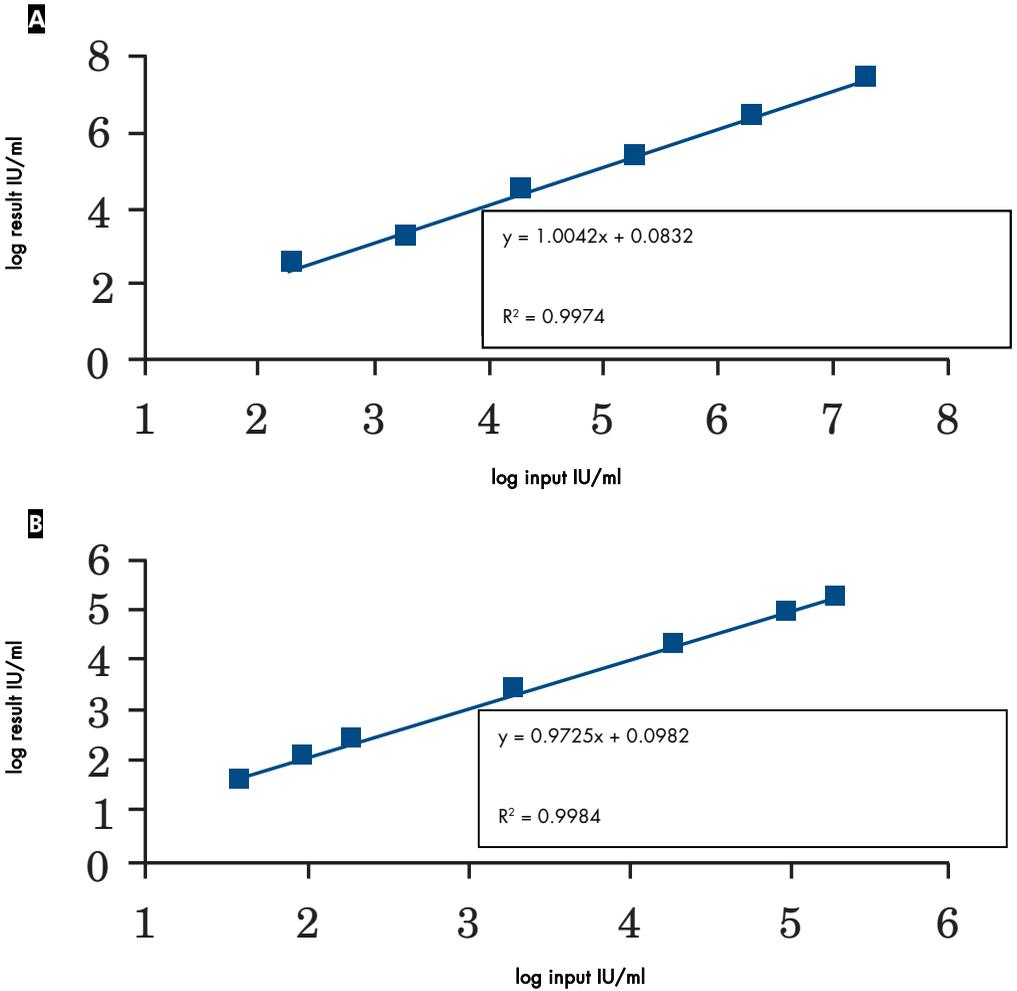


Figure 1. Linear range of the QIAamp DSP Virus Procedure using TaqMan assays. The linear range of the QIAamp DSP Virus procedure at 60 μ l elution volume was determined using TaqMan assays for **A** HIV RNA and **B** HBV DNA.

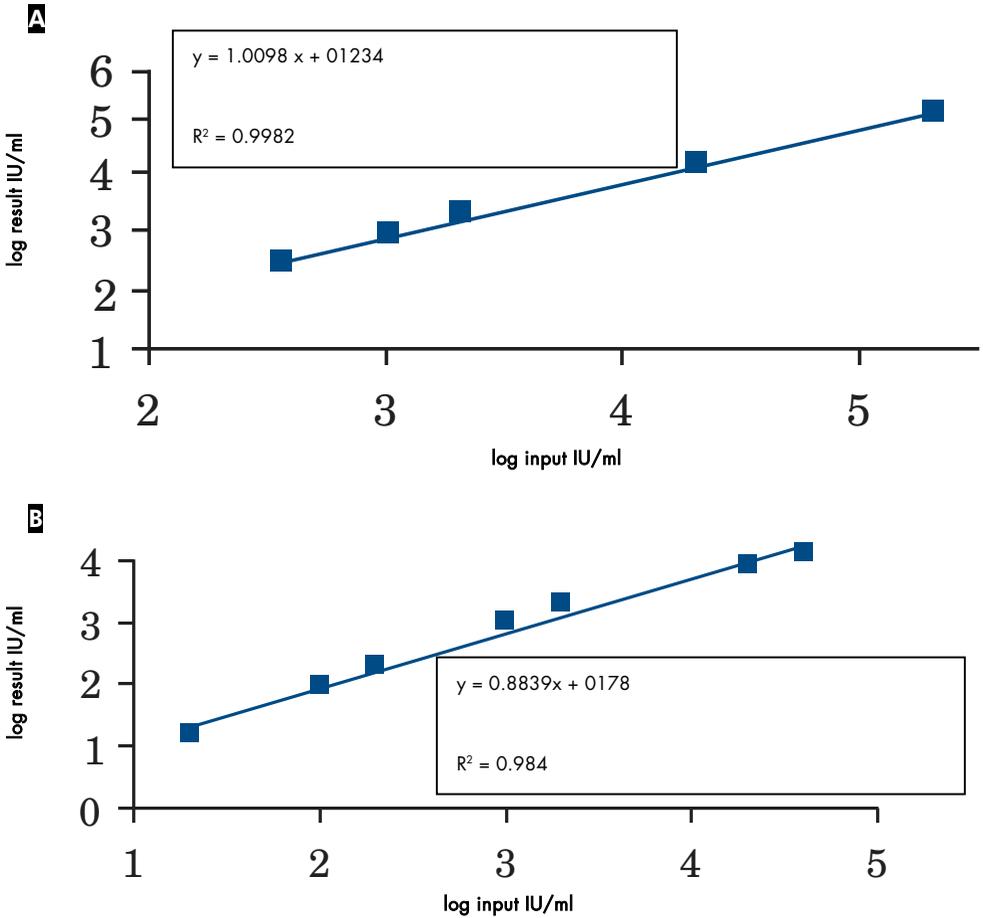


Figure 2. Linear range of the QIAamp DSP Virus Procedure using cobas AMPLICOR MONITOR tests. The linear range of the QIAamp DSP Virus procedure at 60 μ l elution volume was determined using cobas AMPLICOR MONITOR Tests for **A** HIV RNA and **B** HBV DNA.

The detection limit (DL) and quantification limit (QL), according to ICH guidelines 2QA and 2QB, have been determined for the QIAamp DSP Virus procedure (with a starting sample volume of 500 µl and elution volumes of 20 µl and 60 µl) using various downstream diagnostic assays (and).

Table 2.-Detection Limit of the QIAamp DSP Virus Procedure

Assay	Elution volume	95% cut off
<i>artus</i> [®] RealArt™ HBV DNA	20 µl	2.31 IU/ml (n=240)
<i>artus</i> RealArt HCV RNA	20 µl	24.31 IU/ml (n=192)
AMPLICOR manual HIV RNA	60 µl	90.92 IU/ml (n=209)
TaqMan HBV DNA	60 µl	4.73 IU/ml (n=192)

Table 3.-Quantification Limit of the QIAamp DSP Virus Procedure

Assay	QL	CV
TaqMan HBV DNA	5.7 IU/ml	< 70% (n=88)
TaqMan HIV RNA	52 IU/ml	< 60% (n=88)
cobas AMPLICOR HIV RNA	100 IU/ml	< 60% (n=88)
cobas AMPLICOR HBV DNA	30 IU/ml	< 60% (n=88)
cobas AMPLICOR HCV RNA [®]	700 IU/ml	< 60% (n=66)

QIAamp DSP Virus Procedure

Sample



Lyse



Bind

Vacuum



Wash
(AW1)

Remove EXT
before vacuum
is applied

Vacuum



Wash
(AW2)

Vacuum



Wash
(ethanol)

Vacuum



Dry spin

Elute



Pure viral nucleic acids

Read the protocol (page 27) carefully before starting.

Into LT, add 75 μ l QP, 500 μ l sample, and 500 μ l AL.

Vortex 15 seconds.

Incubate 15 minutes (\pm 1 min) at 56°C (\pm 1°C).

Add 600 μ l ethanol.

Vortex 15 seconds.

Incubate 5 min (\pm 1 minute) at room temperature (15–25°C).

Transfer lysate into QIAamp MinElute Column with attached EXT.

Add 600 μ l reconstituted AW1.

Remove EXT.

Add 700 μ l reconstituted AW2.

Add 750 μ l ethanol.

Place QIAamp MinElute Column in WT.

Centrifuge 1 minute at 14,000 rpm.

Place QIAamp MinElute Column in WT.

Incubate 3 minutes at 56°C.

Place QIAamp MinElute Column in ET.

Add 20 μ l or 60 μ l AVE.

Incubate 3 minutes at room temperature .

Centrifuge 1 minute at 14,000 rpm.

Equipment and Reagents to Be Supplied by User

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

- Ethanol (96–100%)
- Pipets* and pipet tips (to prevent cross-contamination, we strongly recommend the use of pipet tips with aerosol barriers)
- Disposable gloves
- Heating block* for lysis of samples at 56°C (we recommend the Eppendorf® Thermomixer comfort with thermoblock for 2.0 ml micro test tubes[†])
- Microcentrifuge*
- Measuring cylinder (50 ml)
- Vortexer
- QIAvac 24 Plus vacuum system (QIAvac 24 Plus, cat. no. 19413, QIAvac Connecting System, cat. no. 19419 and Vacuum Pump, cat. no. 84020[§]), or an equivalent general laboratory vacuum system

* To ensure that samples are properly processed in the QIAamp DSP Virus procedure, we strongly recommend that instruments (e.g., pipets and heating blocks) are calibrated according to the manufacturers' recommendations.

[†] This is not a complete list of suppliers and does not include many important vendors of biological supplies.

[§] The cat. no. 84020 refers to a pump suitable for European countries (e.g., Germany). For countries with other requirements for voltage or plugs, contact QIAGEN Technical Service.

Important Notes

Important points before starting

- After receiving the kit, check the kit components for damage. If the blister packs or the buffer bottles are damaged, contact QIAGEN Technical Services or your local distributor. In case of liquid spillage, refer to “Warnings and Precautions” (page 8).
- Do not use damaged kit components, since their use may lead to poor kit performance.
- Always use RNase-free equipment.
- Store ethanol (96–100%) on ice during the procedure.
- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature (15–25°C).
- Always use disposable gloves and regularly check that they are not contaminated with sample material.
- Discard gloves if they become contaminated, and at least at all steps marked with the glove symbol. 
- To avoid cross-contamination, open only one tube at a time.
- Do not use kit components from other kits with the kit you are currently using, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To ensure safety from potentially infectious material, we recommend working under laminar air-flow conditions until the samples are lysed.
- This kit should only be used by personnel trained in in vitro diagnostic laboratory practice.

- The procedure provides instructions for processing a single plasma or serum sample. However, up to 24 samples can be processed at the same time on the QIAvac 24 Plus vacuum system.

Preparing RNA

When preparing viral RNA, work quickly during the manual steps of the procedure.

Elution Buffer (AVE) contains sodium azide‡, an antimicrobial agent that prevents growth of RNase-producing organisms. However, as this buffer does not contain any RNase-degrading chemicals, it will not actively inhibit RNases introduced by inappropriate handling. Extreme care should be taken to avoid contamination with RNases when handling Elution Buffer (AVE).

Storing samples

After collection and centrifugation, plasma or serum can be stored at 2–8°C for up to 6 hours. For long-term storage, freezing at –20°C or –80°C in aliquots is recommended. Frozen plasma or serum samples must not be thawed more than once. Repeated freeze–thawing leads to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral nucleic acids. In addition, cryoprecipitates formed during freeze–thawing will clog the QIAamp MinElute Column membrane. If cryoprecipitates are visible, they should be pelleted by centrifugation at approximately 6800 x g for 3 minutes. The cleared supernatant should be aspirated and processed immediately without disturbing the pellet.

‡ When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Preparing reagents and buffers

Preparing QIAGEN Protease

Add the entire contents of the vial containing 4.4 ml Protease Solvent (PS) to the vial of lyophilized QIAGEN Protease (QP) and mix carefully. To avoid foaming, mix by inverting the vial several times. Ensure that the QIAGEN Protease (QP) is completely dissolved.



Do not add QIAGEN Protease (QP) directly to Lysis Buffer (AL).

Adding Carrier RNA and internal control to Lysis Buffer

Carrier RNA serves two purposes. Firstly, it enhances binding of viral nucleic acids to the QIAamp MinElute Column membrane, especially if there are very few target molecules in the sample. Secondly, the addition of large amounts of Carrier RNA reduces the chance of viral RNA degradation in the rare event that RNase molecules are not denatured by the chaotropic salts and detergent in Lysis Buffer (AL). If Carrier RNA is not added to Lysis Buffer (AL), this may lead to reduced viral RNA or DNA recovery.

Carrier RNA may also be included in some internal control reagents of commercial downstream assays. In these cases, please refer to the relevant instructions for use from the manufacturer of the downstream assay.

Use of an internal control is strongly recommended when using the QIAamp DSP Virus Kit in combination with diagnostic amplification systems. Internal control RNA or DNA and reconstituted Carrier RNA should be added to Lysis Buffer (AL), and mixed thoroughly by inverting the tube 10 times. To avoid foaming, do not vortex.

Refer to the manufacturer's instructions to determine the optimal concentration of internal control. Using a concentration other than that recommended may result in incorrect results. When calculating the correct amount of internal control to use, take into consideration the

starting volume of the sample and the elution volume. Remember that the QIAamp DSP Virus Kit uses a starting sample volume of 500 μl .

To prepare the Carrier RNA solution, add 310 μl Elution Buffer (AVE) to the tube containing 310 μg lyophilized Carrier RNA to obtain a solution of 1 $\mu\text{g}/\mu\text{l}$. Dissolve the Carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at -20°C . Do not freeze-thaw aliquots of Carrier RNA more than 2 times.

Note that Carrier RNA does not dissolve in Lysis Buffer (AL). It must first be dissolved in Elution Buffer (AVE) and then added to Lysis Buffer (AL). Ensure that the Carrier RNA is completely dissolved in the correct volume of Elution Buffer (AVE) before mixing it with Lysis Buffer (AL).



Always use the correct internal control for the downstream assay. Refer to manufacturers' instructions for further information.

Calculate the volume of Lysis Buffer (AL)/Carrier RNA mix needed per batch of samples by selecting the number of samples to be simultaneously processed from Table 4. Volumes are calculated using the following sample calculation:

$$n \times 0.55 \text{ ml} = y \text{ ml}$$

$$y \text{ ml} \times 11.2 \mu\text{l}/\text{ml} = z \mu\text{l}$$

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

y = calculated volume of Lysis Buffer (AL)

z = volume of Carrier RNA/Elution Buffer (AVE) to add to Lysis Buffer (AL)

Table 4.-Volumes of Lysis Buffer (AL) and Carrier RNA/Elution Buffer (AVE) Required for the QIAamp DSP Virus Procedure

No. samples	Vol. AL (ml)	Vol. Carrier RNA/AVE (µl)	No. samples	Vol. AL (ml)	Vol. Carrier RNA/AVE (µl)
1	0.55	6.2	13	7.15	80.0
2	1.10	12.3	14	7.70	86.0
3	1.65	18.5	15	8.25	92.4
4	2.20	24.6	16	8.80	98.6
5	2.75	30.8	17	9.35	104.7
6	3.30	37.0	18	9.90	110.9
7	3.85	43.1	19	10.45	117.0
8	4.40	49.3	20	11.00	123.2
9	4.95	55.0	21	11.55	129.4
10	5.50	61.6	22	12.10	135.5
11	6.05	67.8	23	12.65	141.7
12	6.60	73.9	24	13.20	147.8

Preparing Wash Buffer 1 (AW1)

Using a measuring cylinder, add 25 ml ethanol (96–100%) to the bottle containing 19 ml Wash Buffer 1 (AW1) concentrate. Store the reconstituted Wash Buffer 1 (AW1) at room temperature (15–25°C).



Always mix the reconstituted Wash Buffer 1 (AW1) by inverting the bottle several times before starting the procedure.

Preparing Wash Buffer 2 (AW2)

Using a measuring cylinder, add 30 ml ethanol (96–100%) to the bottle containing 13 ml Wash Buffer 2 (AW2) concentrate. Store the reconstituted Wash Buffer 2 (AW2) at room temperature (15–25°C).



Always mix the reconstituted Wash Buffer 2 (AW2) by inverting the bottle several times before starting the procedure.

Preparing Elution Buffer (AVE)

Four tubes of Elution Buffer (AVE) are provided with the kit. Take care not to contaminate the buffer with RNases. If performing 4 purification procedures or less using a single kit, we recommend discarding the tube of Elution Buffer (AVE) at the end of each procedure.

Eluting viral nucleic acids

For downstream applications that require small starting volumes (e.g., some PCR and RT-PCR assays), using viral nucleic acids eluted in 20 µl Elution Buffer (AVE) may increase assay sensitivity.

The volume of viral nucleic acids eluted from a QIAamp MinElute Column can be up to 5 µl less than the volume of Elution Buffer (AVE) applied to the column. For example, eluting viral nucleic acids with 60 µl Elution Buffer (AVE) leads to an eluate of approximately 55 µl, while eluting with 20 µl results in approximately 15 µl of eluate.

The volume of eluate recovered depends on the nature of the sample. If the volume of eluate recovered is too low for the downstream assay, increase the volume by adding more Elution Buffer (AVE).

Eluted viral nucleic acids are collected in Elution Tubes (ET). If storing the viral nucleic acids for up to 24 hours, we recommend storage at 2–8°C.

Yield and quality of viral nucleic acids

The yield and quality of the isolated viral nucleic acids are suitable for all types of downstream detection procedures in molecular diagnostics. Diagnostic assays should be performed according to the manufacturers' instructions.

Setting up the QIAvac 24 Plus vacuum system

Ensure that you set up the Column Extender (EXT), QIAamp MinElute Column, VacConnector (VC) and VacValve correctly (see Figure 3).

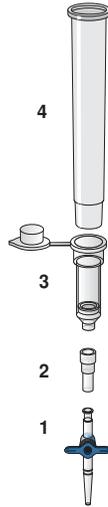


Figure 3. Assembly of components of the QIAamp DSP Virus Kit for vacuum processing of samples:

1: VacValve (provided with the vacuum system)

3: QIAamp MinElute Column

2: VacConnector (VC)

4: Column Extender (EXT)

We recommend labeling the Lysis Tubes (LT), Elution Tubes (ET) and the QIAamp MinElute Columns for use on the QIAvac 24 Plus vacuum system according to the scheme in Figure 4 to avoid the mix-up of samples. This figure can be photocopied and labeled with the names of the samples.

Date: _____

Operator: _____

Run ID: _____

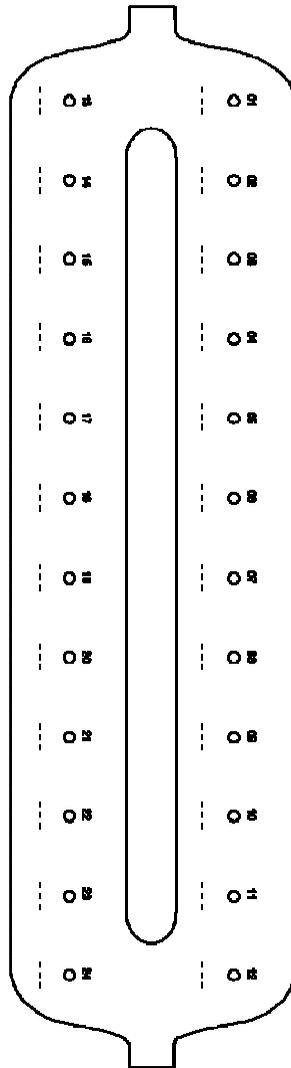


Figure 4. Labeling scheme for Lysis Tubes (LT), Elution Tubes (ET) and QIAamp MinElute Columns for use on the QIAvac 24 Plus vacuum system.

Protocol: Isolation and Purification of Viral Nucleic Acids from Plasma and Serum

For isolation and purification of viral nucleic acids from 500 µl of EDTA- or citrate-treated plasma and serum.

Things to do before starting

- Equilibrate samples to room temperature (15–25°C), and ensure that they are well mixed.
- Add Carrier RNA reconstituted in Elution Buffer (AVE) or internal control to Lysis Buffer (AL), according to the instructions on page 19.
- Ensure that Wash Buffer 1 (AW1), Wash Buffer 2 (AW2) and QIAGEN Protease (QP) have been prepared according to the instructions in “Important Notes” on page 17.
- Equilibrate Elution Buffer (AVE) to room temperature (15–25°C) for use in step 18. If possible, use fresh Elution Buffer (AVE) for each procedure (4 tubes are provided).
- Set a heating block to 56°C for use in steps 4 and 17.
- To avoid cross-contamination, insert a VacConnector (VC) into each luer adapter of the vacuum system.
- Ensure that the waste bottle of the vacuum system is empty and all couplings are connected correctly.
- For details about operation of the vacuum system, especially maintenance, refer to the handbook supplied with it.

Procedure

1. Pipet 75 µl QIAGEN Protease (QP) into a Lysis Tube (LT).



Check the expiration date of the reconstituted protease before use.

2. Add 500 μl plasma or serum to the Lysis Tube (LT).
3. Add 500 μl Lysis Buffer (AL) (containing 11.2 $\mu\text{g}/\text{ml}$ Carrier RNA) to the Lysis Tube (LT), close the lid, and mix by pulse-vortexing for 15 seconds.

To ensure efficient lysis, it is essential that the sample and Lysis Buffer (AL) are mixed thoroughly to yield a homogeneous solution.



Lysis Buffer (AL) contains internal control. Since Lysis Buffer (AL) has a high viscosity, be sure to add the correct volume of Lysis Buffer (AL) by pipetting carefully or by using a suitable pipet such as an Eppendorf multistep pipet or equivalent.



Do not add QIAGEN Protease (QP) directly to Lysis Buffer (AL).

4. Incubate at 56°C ($\pm 1^\circ\text{C}$) for 15 minutes (± 1 min).
5. Centrifuge the Lysis Tube (LT) for ≥ 5 seconds at full speed to remove drops from the inside of the lid.



6. Change gloves and open the Lysis Tube (LT) carefully.

7. Add 600 μl ethanol (96–100%) to the Lysis Tube (LT), close the lid, and mix thoroughly by pulse-vortexing for ≥ 15 seconds. Incubate for 5 minutes (± 1 minute) at room temperature (15–25°C).

8. Centrifuge the Lysis Tube (LT) for ≥ 5 seconds at full speed to remove drops from the inside of the lid.

9. Insert the QIAamp MinElute Column into the VacConnector (VC) on the vacuum system (see Figure 3, page 24). Insert a Column Extender (EXT) into the open QIAamp MinElute Column.



Keep the Wash Tube (WT) for the dry spin in step 16.



10. Change gloves and open only one tube at a time.

11. Carefully apply the entire lysate from step 7 into the Column Extender (EXT) of the QIAamp MinElute Column without wetting the rim. Avoid touching the QIAamp MinElute Column membrane with the pipet tip.

12. Switch on the vacuum pump. After the lysate has been drawn through the QIAamp MinElute Column, open the valve of the vacuum system, and release the vacuum.

If processing several QIAamp MinElute Columns at the same time, we recommend closing the VacValve of each column after lysate has passed through to reduce the duration of this vacuum step.



If the lysate has not completely passed through the membrane after 15 minutes, discard the QIAamp MinElute Column and repeat the procedure with a new sample.



The vacuum system valve should be used for rapid release of the vacuum pressure.

13. Apply 600 μ l Wash Buffer 1 (AW1) to the QIAamp MinElute Column. Carefully remove and discard the Column Extender (EXT), and close the valve of the vacuum system. After Wash Buffer 1 (AW1) has been drawn through the QIAamp MinElute Column, open the valve, and release the vacuum.



To avoid cross-contamination, ensure that removed Column Extenders (EXT) do not pass over neighboring QIAamp MinElute Columns.

14. Apply 750 μ l Wash Buffer 2 (AW2) to the QIAamp MinElute Column without wetting the rim. Avoid touching the QIAamp MinElute Column membrane with the pipet tip. Leave the lid of the column open, and close the valve of the vacuum system. After Wash Buffer 2 (AW2) has been drawn through the QIAamp MinElute Column, open the valve, and release the vacuum.

15. Apply 750 μ l ethanol (96–100%) to the QIAamp MinElute Column without wetting the rim. Avoid touching the QIAamp MinElute Column membrane with the pipet tip. Leave the lid of the column open, and close the valve of the vacuum system. After ethanol has been drawn through the QIAamp MinElute Column, open the valve, and release the vacuum.



Use aerosol-barrier pipet tips to apply ethanol to the QIAamp MinElute Column.

16. Close the lid of the QIAamp MinElute Column, remove it from the vacuum system, and discard the VacConnector (VC). Place the QIAamp MinElute Column in the Wash Tube (WT) saved from step 9, and centrifuge at full speed (approximately 20,000 \times g, or 14,000 rpm) for 1 minute to dry the membrane completely. Discard the Wash Tube (WT) containing the filtrate.



Omission of the dry centrifugation might lead to inhibition of the downstream assay.

17. Place the QIAamp MinElute Column in a new Wash Tube (WT), and incubate with the lid open at 56°C for 3 minutes to evaporate any remaining liquid.

18. Place the QIAamp MinElute Column in a clean Elution Tube (ET), and discard the Wash Tube (WT). Carefully open the lid of the QIAamp MinElute Column, and apply 20 μ l or 60 μ l Elution Buffer (AVE) (depending on the downstream assay) to the center of the membrane. Close the lid and incubate at room temperature (15–25°C) for \geq 3 minutes. Centrifuge at full speed (approximately 20,000 \times g, or 14,000 rpm) for 1 minute to elute the viral nucleic acids.



Follow the maintenance procedure for the vacuum system after performing this protocol (see the handbook supplied with the vacuum system for more details).

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Revision History

Document Revision History	
R3	Added clarifying footnote about Vacuum Pump cat. no., see page 16.
08/2018	Updated warnings and precautions. Updated format of handbook.

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