June 2022

QIAamp® DSP Virus Kit Instructions for Use (Handbook)

Σ 50

Version 2

IVD

For In Vitro Diagnostic Use

For use with QIAamp® DSP Virus Kit

CE

REF 60704

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R1 MAT 1127541

Sample to Insight

Contents

Intended Use

The QIAamp® DSP Virus Kit is intended for manual isolation and purification of viral nucleic acids from human plasma or serum samples.

The QIAamp DSP Virus Kit utilizes silica-membrane technology (QIAamp technology) for isolation and purification of viral nucleic acids from human plasma or serum samples.

The product is intended for in vitro diagnostic use and to be used by professional users, such as technicians and physicians who are trained in molecular biological techniques.

Intended User

The product is intended for use by professional users such as technicians and physicians trained in molecular biological techniques.

Description and Principle

The QIAamp DSP Virus procedure comprises 4 steps (lyse, bind, wash, and elute) and is carried out using QIAamp MinElute® columns together with a vacuum manifold and a standard microcentrifuge. The procedure is designed to minimize the potential for sample-to-sample cross-contamination and allows safe handling of potentially infectious samples. The simple QIAamp DSP Virus procedure is suitable for simultaneous processing of multiple samples. The QIAamp DSP Virus Kit can be used for isolation of viral RNA and DNA from a broad range of RNA and DNA viruses. However, performance characteristics for every virus species have not been established and must be validated by the user.

Lysis with QIAGEN Protease (QP)

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.

Adsorption to the QIAamp MinElute membrane

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

Removing residual contaminants

Nucleic acids remain bound to the membrane, while contaminants are efficiently washed away during 3 wash steps.

Elution of viral nucleic acids

In a single step, highly pure viral RNA and DNA are eluted from the QIAamp MinElute column membrane in Elution Buffer (AVE), equilibrated to room temperature. The QIAamp MinElute columns allow elution volumes of 20 µl or 60 µl. 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.

For downstream applications that require a larger starting volume, the elution volume can be increased up to 60 µl. However, an increase in elution volume will decrease the concentration of nucleic acids in the eluate.

Due to remaining elution buffer retained by the spin column membrane after centrifugation, the eluate volume recovered can be lower than the volume of elution buffer applied to the column. Furthermore, the volume of eluate recovered depends on the nature of the sample.

Eluted viral nucleic acids are collected in Elution Tubes (ET) and can be stored at 2–8°C for up to 24 hours. For long term storage over 24 hours, we recommend storing purified nucleic acids at –20°C.

Note: Eluate stability highly depends on various factors and relates to the specific downstream application. It has been evaluated for the QIAamp DSP Virus Kit in conjunction with exemplary downstream applications. It is the responsibility of the user to consult the instructions for use of the specific downstream application used in their laboratory and/or validate the whole workflow to establish appropriate storage conditions.

Yield and quality of viral nucleic acids

Yields of viral nucleic acid isolated from biological samples are normally below 1 µg. Quantitative amplification methods are recommended for determination of yields. When

quantifying nucleic acids isolated using the QIAamp DSP Virus protocol, remember that there will be considerably more carrier RNA in the sample than viral RNA.

Carrier RNA serves two purposes: First, it enhances binding of viral nucleic acids to the QIAamp 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 viral RNA degradation in the rare event that RNase molecules escape denaturation 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.

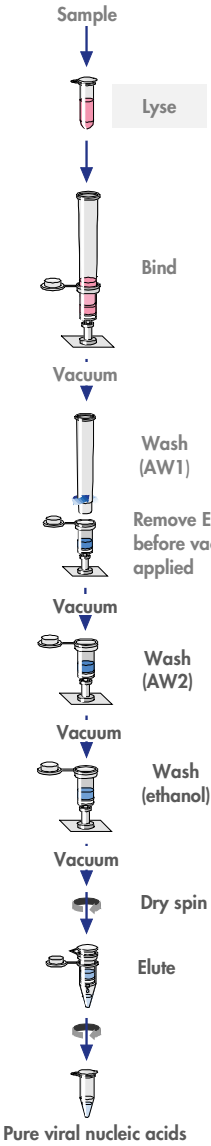
Different amplification systems vary in efficiency depending on the total amount of nucleic acids present in the reaction. Eluates from this kit contain both viral nucleic acids and carrier RNA, and amounts of carrier RNA will greatly exceed amounts of viral nucleic acids. Calculations of how much eluate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

Addition of internal controls

Using the QIAamp DSP Virus protocol in combination with commercially available amplification systems may require the introduction of an internal control into the purification procedure. Internal control RNA or DNA should be added together with the carrier RNA to the lysis buffer. For optimal purification efficiency, internal control molecules should be longer than 200 nucleotides, as smaller molecules are not efficiently recovered.

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

QIAamp DSP Virus Procedure



Read the protocol (page 30) carefully before starting.
Into LT, add 75 µl QP, 500 µl sample, and 500 µl AL.
Vortex 15 seconds.
Incubate 15 minutes at 56°C.
Add 600 µl ethanol.
Vortex 15 seconds.
Incubate 5 min at room temperature (15–25°C).

Transfer lysate into QIAamp MinElute column with attached EXT.

Add 600 µl reconstituted AW1.

Remove EXT.

Add 750 µl reconstituted AW2.

Add 750 µl 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 µl or 60 µl AVE.
Incubate 3 minutes at room temperature.
Centrifuge 1 minute at 14,000 rpm.

Summary and explanation

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.

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

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 for later use.

Materials Provided

Kit contents

QIAamp DSP Virus Catalog no. Number of preps			60704 50
QIAamp MinElute®	QIAamp MinElute columns with Wash Tube (WT)s (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 (WT)s (2 ml)	WASH TUBE	50
AL	Lysis Buffer*	LYS BUF	33 ml
AW1	Wash Buffer 1 (AW1)* (concentrate)	WASH BUF 1 CON	19 ml
AW2	Wash Buffer 2 (AW2)† (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
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* Contains guanidine hydrochloride. Not compatible with disinfectants containing bleach. See page 14 for safety information.

† Contains sodium azide as a preservative

‡ Resuspension volume 4.4 ml

Components of the kit

The principal components of the kit containing active ingredients are explained below.

Reagent	Active ingredients	Concentration (w/w) [%]
QIAGEN Protease (QP)	Subtilisine	≥ 90 to ≤ 100
AL	Guanidine hydrochloride Maleic acid	≥ 30 to < 50 ≥ 0.1 to < 1
AW1	Guanidine hydrochloride	≥ 50 to < 70

Materials Required but Not Provided

Additional reagents

- Ethanol (96–100%)*

Consumables

- Pipettes† and pipette tips (to prevent cross-contamination, we strongly recommend the use of pipette tips with aerosol barriers)
- Disposable gloves

Equipment

- Heating block† for lysis of samples at 56°C for 2.0 ml micro test tubes
- Microcentrifuge†
- Measuring cylinder (50 ml)
- Vortexer
- QIAvac 24 Plus vacuum system (cat. no. 19413) or equivalent†

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
† Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Warnings and Precautions

Please be aware that you may be required to consult your local regulations for reporting serious incidents that have occurred in relation to the device to the manufacturer and/or its authorized representative and the regulatory authority in which the user and/or the patient is established.

For In Vitro Diagnostic Use.

Read all instructions carefully before using the kit.

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 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.
- Specimens and samples are potentially infectious. Discard sample and assay waste according to your local safety procedures.

Emergency information

CHEMTREC
USA & Canada 1-800-424-9300
Outside USA & Canada +1 703-527-3887

Precautions

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

Lysis 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. Call a POISON CENTER or doctor/physician if you feel unwell. If skin irritation or rash occurs: Get medical advice/attention. Take off contaminated clothing and wash before reuse. Dispose of contents/container to an approved waste disposal plant.

Wash Buffer 1 (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. Take off contaminated clothing and wash before reuse. Dispose of contents/container to an approved waste disposal plant.

QIAGEN Protease (QP)

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 person to fresh air and keep comfortable for breathing.

Disposal

The waste contains samples and reagents. This waste may contain toxic or infectious material and must be disposed properly. Refer to your local safety regulations for proper disposal procedures.

For more information, please consult the appropriate safety data sheets (SDSs). These are available online in PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Reagent Storage and Handling

Attention should be paid to expiration dates and storage conditions printed on the box and labels of all components. Do not use expired or incorrectly stored components.

QIAamp MinElute columns should be stored at 2–8°C upon arrival. When stored properly, the QIAamp MinElute columns are stable until the expiration date on the kit box.

Note: To ensure that kit components from different kits are not mixed, please label the QIAamp MinElute columns with the respective kit lot number.

All buffers can be stored at room temperature (15–25°C) until the expiration date on the kit box.

Lyophilized carrier RNA can be stored at room temperature until the expiration date on the kit box.

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

In-use stability

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 24. 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.

QIAGEN Protease (QP) reconstituted in Protease Solvent (PS) is stable for up to 1 year when stored at 2–8°C, but only until the expiration date. Keeping the QIAGEN Protease (QP) stock solution at room temperature for prolonged periods of time should be avoided.

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 on the kit box.

Specimen Collection, Storage, and Handling

Note: Sample stability highly depends on various factors and relates to the specific downstream application. It has been evaluated in conjunction with exemplary downstream applications. It is the responsibility of the user to consult the instructions for use of the specific downstream application used in their laboratory and/or validate the whole workflow to establish appropriate storage conditions.

For general collection, transport and storage recommendations refer to the approved CLSI guideline MM13-A "Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods". Furthermore, the manufacturer's instructions for the selected sample collection device shall be followed during sample preparation, storage, transport, and general handling.

The purification procedure is optimized for use with human plasma and serum samples. Blood samples treated with EDTA or citrate as anticoagulant can be used for plasma preparation. Samples can be either fresh or frozen, provided that they have not been frozen and thawed more than once. Thaw frozen samples with mild agitation to ensure thorough mixing.

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 –80°C to –20°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. Start the purification procedure immediately. Centrifugation at low g-forces does not reduce viral titers.

Note: According to exemplary interference studies for the QIAamp DSP Virus Kit and in line with ISO 20186-2:2019(E), heparin from blood collection tubes may impact the purity of the isolated nucleic acids and possible carryover into eluates may cause inhibitions in some downstream applications. Therefore, we recommend usage of blood samples treated with EDTA or citrate as anticoagulant.

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 14). Do not use damaged kit components, since their use may lead to poor kit performance.
- Always use RNase-free equipment.
- Always change pipette tips between liquid transfers. To minimize cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- 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 minimize cross-contamination, open only one tube at a time.
- After all pulse-vortexing steps, briefly centrifuge the microcentrifuge tubes to remove drops from the inside of the lid.
- All centrifugation steps are carried out at room temperature (15–25°C).
- The user should ensure that traceability of the samples is kept during the entire procedure.
- 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 minimize the risk of infection from potentially infectious material, we recommend working under laminar air-flow conditions until the samples are lysed.
- 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.

- This kit should only be used by personnel trained in in vitro diagnostic laboratory practice.

Handling of QIAamp MinElute columns

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

- Carefully apply the sample or solution to the QIAamp MinElute column. Pipet the sample into the QIAamp MinElute column without wetting the rim of the column.
- Always change pipette tips between all liquid transfers. The use of aerosol-barrier pipette tips is recommended.
- Avoid touching the QIAamp MinElute membrane with the pipette tip.
- Open only one QIAamp MinElute column at a time, and take care to avoid generating aerosols.

Preparing reagents and buffers

Preparing RNA

When preparing viral RNA, work quickly during the manual steps of the procedure and read the Appendix on page 45 before starting.

Preparing QIAGEN Protease (QP)

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 (AL)*

Use of an internal control is strongly recommended when using the QIAamp DSP Virus Kit in combination with diagnostic amplification systems. Refer to manufacturers' instructions for further information. Internal control and reconstituted carrier RNA should be added to Lysis Buffer (AL), and mixed gently by inverting the tube 10 times. To avoid foaming, do not vortex. If internal control is used, reduce volume for Lysis Buffer (AL) accordingly (see Table 1 for further details).

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 µg/µl. 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 3 times.

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).

* Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 14 for safety 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 1. Volumes are calculated using the following sample calculation:

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

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

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)

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

Table 1. 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

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 amplification system, transfer only the required amount of dissolved carrier RNA to the tubes containing Lysis Buffer (AL). For each microgram of carrier RNA required per preparation, add 5 µl Buffer AVE-dissolved carrier RNA per milliliter of Lysis Buffer (AL). Use of less than 5.6 µg carrier RNA per sample must be validated for each particular sample type and downstream assay.

* If internal control is used, reduce volume for Lysis Buffer (AL) accordingly.

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. Tick the checkbox on the label to indicate that ethanol has been added. 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. Tick the checkbox on the label to indicate that ethanol has been added. 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.

* Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 14 for safety information.

† Contains sodium azide as a preservative.

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 1).

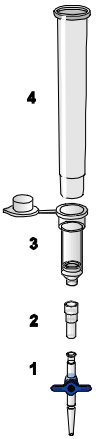


Figure 1. Assembly of components of the QIAamp DSP Virus Kit for vacuum processing of samples:

1. VacValve (provided with the vacuum system)
2. VacConnector (VC)
3. QIAamp MinElute column
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 2 to avoid the mix-up of samples. This figure can be photocopied and labeled with the names of the samples.

Date: _____

Operator: _____

Run ID: _____

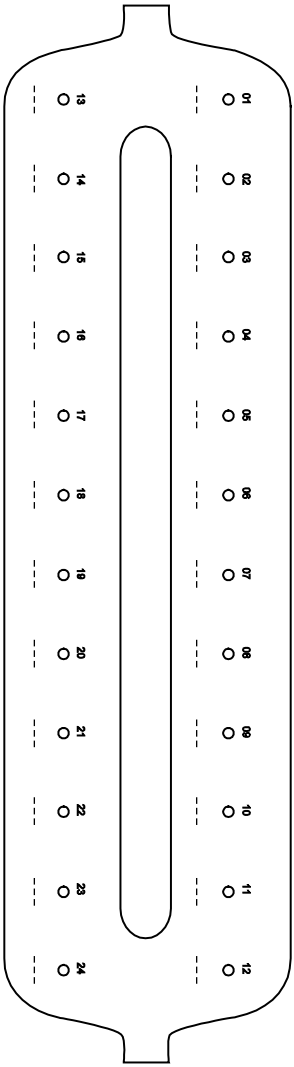


Figure 2. Labeling scheme for Lysis Tubes (LT), Elution Tubes (ET) and QIAamp MinElute columns for use on the QIAvac 24 Plus vacuum system.

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.
- Make sure that all reagents and the QIAamp MinElute columns (in closed blisters) are equilibrated to room temperature.
- Set a heating block to 56°C for use in steps 4 and 17.
- Ensure that Wash Buffer 1 (AW1), Wash Buffer 2 (AW2) and QIAGEN Protease (QP) have been prepared according to the instructions in "Important points before starting" on page 22.
- If a precipitate has formed in Lysis Buffer (AL), dissolve by incubating at 56°C.
- Add carrier RNA reconstituted in Elution Buffer (AVE) or internal control to Lysis Buffer (AL), according to the instructions on page 24.
- If possible, use fresh Elution Buffer (AVE) for each procedure (4 tubes are provided).
- To minimize cross-contamination, insert a VacConnector (VC) into each luer adapter of the vacuum system.
- Quality control procedures at QIAGEN employ functional kit release testing for each individual kit lot. Therefore, do not mix reagents from different kit lots, and do not combine individual reagents from different reagent lots.
- 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 µg/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.

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

4. Incubate at 56°C for 15 minutes.
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 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 1, page 28). 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.
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 µl 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 µl Wash Buffer 2 (AW2) to the QIAamp MinElute column without wetting the rim. 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 µl ethanol (96–100%) to the QIAamp MinElute column without wetting the rim. 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 pipette 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 *x 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 new Elution Tube (ET), and discard the Wash Tube (WT). Carefully open the lid of the QIAamp MinElute column, and apply 20 µl or 60 µl Elution Buffer (AVE) (depending on the downstream assay) to the center of the membrane.

It is important to use a new elution tube to avoid contamination with residual wash buffers that might lead to inhibition of the downstream assay.

Dispensing the elution buffer on the center of the membrane is especially important for smaller elution volumes to ensure optimal retrieval of nucleic acids and elution buffer.

Elution volume can be adapted according to the requirements of the downstream application. Remember that the recovered eluate volume can be lower than the volume of elution buffer applied to the column due to remaining elution buffer retained by the spin column membrane after centrifugation.

Ensure that the elution buffer is equilibrated to room temperature.

19. Close the lid and incubate at room temperature (15–25°C) for ≥3 minutes. Centrifuge at full speed (approximately 20,000 *x g*, or 14,000 rpm) for 1 minute to elute the viral nucleic acids.

    Orient the elution tube 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).

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

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.

Limitations

The system performance has been established in performance evaluation studies purifying viral nucleic acids from human plasma and serum samples.

It is the user's responsibility to verify system performance for any procedures used in their laboratory, which are not covered by the QIAGEN performance evaluation studies.

To minimize the risk of a negative impact on the diagnostic results, adequate controls for downstream applications should be used. Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

Performance Characteristics

The applicable performance characteristics can be found under the resource tab of the product page on **www.qiagen.com**.

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/or protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
General handling	
a) Clogging of pipette tips during sample transfer	Frozen samples were not mixed properly after thawing. Thaw frozen samples with mild agitation to ensure thorough mixing. Cryoprecipitates formed during freeze–thawing will clog the QIAamp MinElute membrane. In case cryoprecipitates are visible, clear the sample by centrifugation for 5 minutes at 16,000 *x g*.
b) Clogged QIAamp MinElute column	If the flow rate is reduced, vacuum time can be extended. Alternatively, close the VacValve, if used, and carefully remove the column extender–VacConnector–VacValve assembly from the QIAamp MinElute column without losing any of the lysate in the column extender. Remove the QIAamp MinElute column from the vacuum manifold, place it in a 2 ml Wash Tube (WT) and spin it at full speed until sample has completely passed through the membrane. Replace the column extender–VacConnector–VacValve assembly containing the remaining lysate. Switch on the vacuum pump, open the VacValve, and continue to load the remaining lysate. Repeat the above procedure if the QIAamp MinElute column continues to clog. Cryoprecipitates formed during freeze–thawing will clog the QIAamp MinElute column membrane. In case cryoprecipitates are visible, clear the sample by centrifugation for 5 minutes at 16,000 *x g*.

		Comments and suggestions
		Using ice-cooled ethanol during lysis can help to lower the risk of membrane clogging. Furthermore, it is essential to add the buffers for lysis in the correct order described above. Do not add QIAGEN Protease (QP) directly to Lysis Buffer (AL).
c)	Precipitate has formed in Lysis Buffer	Dissolve by incubation of Lysis Buffer (AL) at 56°C.
d)	Variable elution volumes	The volume of eluate recovered depends on the nature of the sample. Due to remaining elution buffer retained by the spin column membrane after centrifugation, the eluate volume recovered can be lower than the volume of elution buffer applied to the column. Apply the elution buffer to the center of the membrane. Dispensing the elution buffer on the center of the membrane is especially important for smaller elution volumes to ensure optimal retrieval of nucleic acids and elution buffer.
e)	Vacuum pressure of ~800 to ~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 MinElute 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.

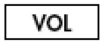
		Comments and suggestions
DNA does not perform well in downstream reactions		
a)	Incomplete sample lysis	If QIAGEN Protease (QP) was subjected to elevated temperature for a prolonged time, it can lose activity. Repeat the procedure using new samples and fresh QIAGEN Protease (QP). Make sure to dissolve QIAGEN Protease (QP) with Protease Solvent according to the instructions above. 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). To ensure efficient lysis, it is essential that the sample and Lysis Buffer (AL) are mixed thoroughly to yield a homogeneous solution. Since Lysis Buffer (AL) has a high viscosity, be sure to add the correct volume of Lysis Buffer (AL) by pipetting carefully and by using a suitable pipette.
b)	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.
c)	Wash Buffer 1 (AW1) or Wash Buffer 2 (AW2) prepared incorrectly	Make sure that the Wash Buffer 1 (AW1) and Wash Buffer 2 (AW2) concentrates were diluted with the correct volume of 96–100% ethanol and mixed by inverting the bottle several times before starting the procedure.

	Comments and suggestions	
d)	Plasma and serum samples were not prepared, stored, or mixed correctly	The purification procedure is optimized for use with human plasma and serum samples. Blood samples treated with EDTA or citrate as anticoagulant can be used for plasma preparation. 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 –80°C to –20°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. Thaw frozen samples with mild agitation to ensure thorough mixing.
e)	Little or no DNA in the eluate	Reduce the elution volume or increase the amount of eluate added to the reaction if possible.
f)	Inappropriate elution volume used	Determine the maximum volume of eluate suitable for your downstream application. Reduce or increase the volume of eluate added to the downstream application accordingly. The elution volume can be adapted proportionally. Elution with smaller volumes of Buffer AVE leads to higher nucleic acid concentrations.
g)	Carryover of potential inhibitor	Be sure to perform dry centrifugation step prior to elution to prevent potential inhibition of the downstream assay. It is important to use a new elution tube to avoid contamination with residual wash buffers that might lead to inhibition of the downstream assay. According to exemplary interference studies for the QIAamp DSP Virus Kit and in line with ISO 20186-2:2019(E), heparin from blood collection tubes may impact the purity of the isolated nucleic acids and possible carryover into eluates may cause inhibitions in some downstream applications. Therefore, we recommend usage of blood samples treated with EDTA or citrate as anticoagulant.

h)	Carrier RNA degraded/prepared incorrectly	Carrier RNA serves two purposes: First, it enhances binding of viral nucleic acids to the QIAamp 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 viral RNA degradation in the rare event that RNase molecules escape denaturation 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 can only be dissolved in Buffer AVE; dissolved carrier RNA should be immediately added to Lysis Buffer (AL). 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.

Symbols

The following symbols appear in the instructions for use or on the packaging and labeling:

Symbol	Symbol definition
 <N>	Contains reagents sufficient for <N> reactions
	Use by
	This product fulfills the requirements of the European Regulation 2017/746 for in vitro diagnostic medical devices.
	In vitro diagnostic medical device
	Catalog number
	Lot number
	Material number (i.e., component labeling)
	Components
	Volume
	Contains
	Number
	Global Trade Item Number
Rn	R is for revision of the Instructions for Use and n is the revision number
	Temperature limitation

Symbol	Symbol definition
	Manufacturer
	Consult instructions for use
	Keep away from sunlight
	Warning/caution
	Upon arrival
	Important note
	Change gloves after protocol step with this mark
	Open on delivery; store QIAamp MinElute columns at 2–8°C
	Write down the current date after adding ethanol to the bottle
	Adding
	Lyophilized

Symbol	Symbol definition
RCNS	Reconstitute in
EtOH	Ethanol
GuHCl	Guanidine hydrochloride
MALEIC ACID	Maleic acid
SUBT	Subtilisin
➡	Leads to
UDI	Unique device identifier

Appendix

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. Care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order 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.

Ordering Information

Product	Contents	Cat. no.
QIAamp DSP Virus Kit (50)	For 50 preps: QIAamp MinElute columns, buffers, reagents, tubes, column extenders, VacConnectors	60704
Accessories		
QIAvac 24 Plus vacuum manifold	Vacuum manifold for processing 1–24 spin columns: QIAvac 24 Plus Vacuum manifold, Luer Plugs, Quick Couplings	19413
Vacuum Pump	Universal vacuum pump	84020
VacConnectors	500 disposable connectors for use with QIAamp spin columns on luer connectors	19407
VacValves	24 valves for use with the QIAvac 24 and QIAvac 24 Plus	19408
Vacuum Regulator	Vacuum Regulator	19530
QIAvac Connecting System	System to connect vacuum manifold with vacuum pump: includes Tray, Waste Bottles, Tubing, Couplings, Valve, Gauge, and 24 VacValves	19419

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

Document Revision History

Revision	Description
R1, June 2022	Version 2, Revision 1 • Update to Kit version 2 for compliance to IVDR • Update of Intended Use & Limitations sections • Update of Description and Principle • Update of Materials Provided (addition of active ingredients) & Material Required but Not Provided • Update of Warnings and Precautions (Addition emergency information & Disposal section) • Update of Reagent Storage and Handling • Update of Specimen Collection, Storage, and Handling • Update of Important Notes & Procedure • Update of Performance Characteristics • Addition of Appendix Section • Addition of Troubleshooting Guide • Update of Symbols section • Addition of Ordering Information

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HB-3032-001