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QIAamp® DSP Virus Spin Kit Instructions for Use (Handbook)

Σ 50

Version 2

IVD

For In Vitro Diagnostic Use

For use with QIAamp® DSP Virus Spin Kit

CE

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QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

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Sample to Insight

Contents

Intended Use 4

Intended User 4

Description and Principle 5

- Lysis with QIAGEN Protease (QP) 5
- Adsorption to the QIAamp MinElute membrane 5
- Removing residual contaminants 6
- Elution of viral nucleic acids 6
- Yield and quality of viral nucleic acids 7
- Addition of internal controls 8
- Automated viral nucleic acid purification on the QIAcube Connect MDx 8
- Summary and explanation 11

Materials Provided 12

- Kit contents 12
- Components of the kit 13

Materials Required but Not Provided 14

- Additional reagents 14
- Consumables 14
- Equipment 14
- For the automated procedure only 14

Warnings and Precautions 16

- Safety information 16
- Emergency information 17

Precautions 18
Disposal 19
Reagent Storage and Handling 20
In-use stability 20
Specimen Collection, Storage, and Handling 22
Important Notes 24
Important points before starting 24
Handling of QIAamp MinElute columns 25
Centrifugation 25
Processing QIAamp MinElute columns in a microcentrifuge 25
Preparing reagents and buffers 26
Protocol: Purification of viral nucleic acids from plasma or serum using a microcentrifuge or the QIAcube Connect MDx 31
Quality Control 35
Limitations 36
Performance Characteristics 37
Troubleshooting Guide 38
Symbols 42
Appendix 45
Ordering Information 46
Document Revision History 48

Intended Use

The QIAamp® DSP Virus Spin Kit is intended for manual or, when used in conjunction with the QIAcube® Connect MDx instrument, for automated isolation and purification of viral nucleic acids from human plasma and serum samples.

The QIAamp DSP Virus Spin Kit utilizes silica-membrane technology (QIAamp technology) for isolation and purification of viral nucleic acids from human plasma and 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 to be used by professional users, such as technicians and physicians that are trained in molecular biological techniques.

Description and Principle

The QIAamp DSP Virus Spin procedure comprises 4 steps (lyse, bind, wash, and elute) and is carried out using QIAamp MinElute® columns in a standard microcentrifuge or automated on the QIAcube Connect MDx. 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 Spin procedure is suitable for simultaneous processing of multiple samples. The QIAamp DSP Virus Spin 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 highly 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 centrifugation. 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.

The 2 ml wash tube (WT)s (provided) complement the QIAamp MinElute column during loading and wash steps.

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 minimal elution volumes of only 20 µl in the manual procedure and 60 µl in the automated procedure. Low elution volume leads to highly concentrated nucleic acid eluates.

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

For downstream applications that require a larger starting volume, the elution volume can be increased up to 150 µl in the manual procedure and up to 100 µl in the automated procedure. 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 nucleic acid are collected in 1.5 ml elution tubes (ET, provided) 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 Spin 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 Spin 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 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 Spin 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.

Automated viral nucleic acid purification on the QIAcube Connect MDx

The QIAcube Connect MDx performs automated isolation and purification of nucleic acids. It can process of up to 12 samples per single run.

If automating the QIAamp DSP Virus Spin Kit on the QIAcube Connect MDx, the instrument may process fewer than 50 samples due to dead volumes, evaporation, and additional reagent consumption by automated pipetting. QIAGEN only guarantees 50 sample preps with manual use of the QIAamp DSP Virus Spin Kit.

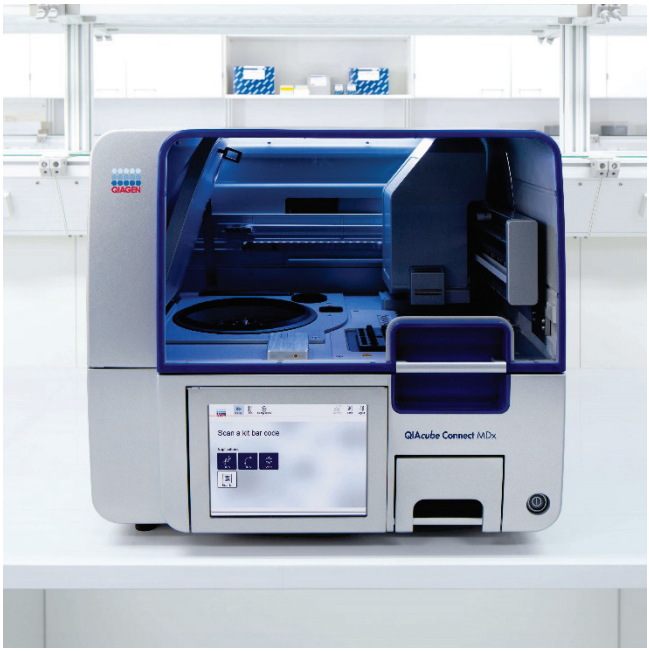
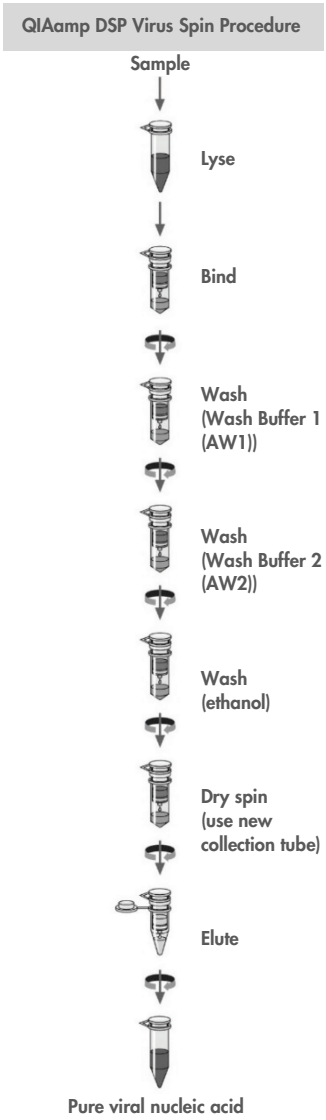


Figure 1. The QIAcube Connect MDx.



Automatable on the QIAcube Connect MDx

Summary and explanation

The QIAamp DSP Virus Spin Kit uses well-established technology for simultaneous purification of viral DNA and RNA. The kit combines the selective binding properties of a silica-based membrane with flexible elution volumes of between 20 and 150 µl in the manual workflow.

The procedure is suitable for use with plasma and serum; either can contain citrate or EDTA. Samples can be either fresh or frozen, provided that 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 simple QIAamp DSP spin procedures are suitable for simultaneous processing of multiple samples. The procedure can be fully automated on the QIAcube Connect MDx (page 9) for increased standardization and ease of use with elution volumes of 60–100 µl in 5 µl increments. The procedure is designed to avoid sample-to-sample cross-contamination and allow safe handling of potentially infectious samples. Viral nucleic acids are eluted in Elution Buffer (AVE), ready for use in amplification reactions (PCR) or storage at –20°C for later use.

Materials Provided

Kit contents

QIAamp DSP Virus Spin Kit Catalog no. Number of preps			61704 50[§]
QIAamp MinElute	QIAamp MinElute columns with Wash tube (WT)s (2 ml)	COL	50
LT	Lysis Tubes (2 ml)	LYS TUBE	50
ET	Elution Tubes (1.5 ml)	ELU TUBE	50
WT	Wash tube (WT)s (2 ml)	WASH TUBE	5 x 50
AL	Lysis Buffer*	LYS BUF	33 ml
AW1	Wash Buffer 1 (AW1)* (concentrate)	WASH BUF 1 CONC	19 ml
AW2	Wash Buffer 2 (AW2)[†] (concentrate)	WASH BUF 2 CONC	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 µg
QP	QIAGEN Protease (QP)[‡]	QPROT	1 vial
–	Instructions for Use (Handbook)		1

* Contains a chaotropic salt. Take appropriate safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. For more information, see page 16.

[†] Contains sodium azide as a preservative.

[‡] See "Preparing reagents and buffers", page 26.

[§] If automating the QIAamp DSP Virus Spin Kit on the QIAcube Connect MDx instrument, the instrument may process fewer than 50 samples due to dead volumes, evaporation, and additional reagent consumption by automated pipetting. QIAGEN only guarantees 50 sample preps with manual use of the QIAamp DSP Virus Spin Kit.

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
- Microcentrifuge† (with rotor for 1.5 ml and 2 ml tubes)
- Measuring cylinder (50 ml)
- Vortexer
- For samples <200 µl: 0.9% NaCl solution

For the automated procedure only

- QIAcube Connect MDx† (cat. no. 9003070)
- Rotor Adapters (cat. no. 990394)
- Rotor Adapter Holder (cat. no. 990392)
- Sample Tubes CB (2 ml, cat. no. 990382, sample input tube)
- Shaker Rack Plugs (cat. no. 9017854)
- Reagent Bottles, 30 ml (cat. no. 990393)

* 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.

- Filter-Tips, 1000 µl (cat. no. 990352)
- Filter-Tips, 1000 µl, wide-bore (cat. no. 990452)
- Filter-Tips, 200 µl (cat. no. 990332)
- SafeSeal Tube, 1.5 ml, Sarstedt® (cat. no. 72.706)

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 directly 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 in order to avoid personal injury or injury to others.

- QIAGEN has not tested the liquid waste generated by QIAamp DSP Virus Spin procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, 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 Spin 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 kit expiration date without affecting performance.

In-use stability

Carrier RNA can only be dissolved in Elution Buffer (AVE); for the manual procedure, dissolved carrier RNA should be immediately added to Lysis Buffer (AL) as described on page 27 only. 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 one year when stored at 2–8°C, but only until the kit 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. For preparation of buffers for the automated procedure, follow the instructions in the *QIAcube Connect MDx User Manual*.

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 –20°C to –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 membrane. If cryoprecipitates are visible, they can be pelleted by centrifugation at approximately 6800 *x g* for 3 minutes. The cleared supernatant should be removed 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 Spin 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 16). 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.
- 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 entire procedure.
- Do not use kit components from other kits with the kits 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.
- For automation, follow the instructions on the user interface (QIAcube Connect MDx) and refer to the appropriate user manual (for QIAcube Connect MDx).
- 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.

Centrifugation

- Wash tube (WT)s and elution tubes for all centrifugation steps are provided together with the kit.
- Centrifugation of QIAamp MinElute columns is performed at approximately 6000 *x g* in order to reduce centrifuge noise. Centrifuging QIAamp MinElute columns at full speed will not affect DNA or RNA yield.
- For the dry spin at the end of the washing procedure and for elution, centrifugation should be carried out at full speed.
- All centrifugation steps should be carried out at room temperature (15–25°C).

Processing QIAamp MinElute columns in a microcentrifuge

- Close the QIAamp MinElute column before placing it in the microcentrifuge. Centrifuge as described.
- Remove the QIAamp MinElute column and wash tube (WT) from the microcentrifuge.

- Place the QIAamp MinElute column in a new wash tube (WT). Discard the filtrate and the wash tube (WT). Please note that the filtrate may contain hazardous waste and should be disposed of appropriately.
- Open only one QIAamp MinElute column at a time, and take care to avoid generating aerosols.

For efficient parallel processing of multiple samples, we recommend filling a rack with wash tube (WT)s so that the QIAamp MinElute columns can be transferred after centrifugation. Used wash tube (WT)s containing the filtrate can be discarded, and the new wash tube (WT)s containing the QIAamp MinElute columns can be placed directly in the microcentrifuge.

Preparing reagents and buffers

Preparation of 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).*

* Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach. See page 16 for safety information.

Adding carrier RNA and internal control to Lysis Buffer (AL)* (for the manual procedure only)

Use of an internal control is strongly recommended when using the QIAamp DSP Virus Spin 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 Spin Kit uses a starting sample volume of 200 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 the 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).

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, page 29. For larger numbers of samples, volumes can be calculated using the sample calculation, below:

$$n \times 0.22 \text{ ml} = y \text{ ml}$$

$$y \text{ ml} \times 28 \text{ µl/ml} = z \text{ µl}$$

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 (Vol.) of Lysis Buffer (AL) and carrier RNA–Elution Buffer (AVE) mix required for specific numbers (No.) of samples for the QIAamp DSP Virus Spin procedure*

No. samples	Vol. Lysis Buffer (AL)* (ml)	Vol. carrier RNA-AVE (µl)	No. samples	Vol. Lysis Buffer (AL)* (ml)	Vol. carrier RNA-AVE (µl)
1	0.22 ml	6.2 µl	13	2.86 ml	80.1 µl
2	0.44 ml	12.3 µl	14	3.08 ml	86.3 µl
3	0.66 ml	18.5 µl	14	3.30 ml	92.4 µl
4	0.88 ml	24.6 µl	16	3.52 ml	98.6 µl
5	1.10 ml	30.8 µl	17	3.74 ml	104.7 µl
6	1.32 ml	37.0 µl	18	3.96 ml	110.9 µl
7	1.54 ml	43.1 µl	19	4.18 ml	117.0 µl
8	1.76 ml	49.3 µl	20	4.40 ml	123.2 µl
9	1.98 ml	55.4 µl	21	4.62 ml	129.4 µl
10	2.20 ml	61.6 µl	22	4.84 ml	135.5 µl
11	2.42 ml	67.8 µl	23	5.06 ml	141.7 µl
12	2.64 ml	73.9 µl	24	5.28 ml	147.8 µl

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 Elution 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.

For the automated procedure, prepare the carrier RNA in AVE as described above (to obtain a solution of 1 µg/µl). In the next step, provide the QIAcube Connect MDx with sufficient carrier RNA solution for the required number of samples plus two additional samples. The required amount is shown on the user interface during loading. The addition of carrier RNA to Lysis Buffer (AL) is done by the QIAcube Connect MDx.

The internal control mixture will be prepared as described on the QIAcube MDx instrument screen. The internal control will be added to the carrier RNA-AVE mixture.

Preparing Wash Buffer 1 (AW1)*

Using a measuring cylinder, add 25 ml of ethanol (96–100%) to a bottle containing 19 ml of Wash Buffer 1 (AW1) concentrate, as described on the bottle. Tick the check box on the label to indicate that ethanol has been added. Store reconstituted Wash Buffer 1 (AW1) at room temperature.

ⓘ Always mix 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 of ethanol (96–100%) to a bottle containing 13 ml of Wash Buffer 2 (AW2) concentrate, as described on the bottle. Tick the check box on the label to indicate that ethanol has been added. Store reconstituted Wash Buffer 2 (AW2) at room temperature.

ⓘ Always mix 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 16 for safety information.

† Contains sodium azide as a preservative.

Protocol: Purification of viral nucleic acids from plasma or serum using a microcentrifuge or the QIAcube Connect MDx

For purification of viral nucleic acids from 200 µl of EDTA- or citrate-treated plasma or serum using the QIAamp DSP Virus Spin Kit using a microcentrifuge or automated on the QIAcube Connect MDx.

Important points before starting

- The procedure below provides instructions for processing a single sample. However, several samples can be processed at the same time; the number depends on the capacity of the microcentrifuge used.
- Automated processing of 2–10 or 12 samples can be performed on the QIAcube Connect MDx.
- For automation, follow the instructions on the user interface (QIAcube Connect MDx) and refer to the QIAcube Connect MDx user manual.

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 step 4 (required for manual procedure and automated procedure with off-board manual lysis).
- Ensure that Wash Buffer 1 (AW1), Wash Buffer 2 (AW2), and QIAGEN Protease (QP) have been prepared according to instructions on pages 26–30.
- If a precipitate has formed in Lysis Buffer (AL), dissolve by incubating at 56°C.

- Add carrier RNA reconstituted in Elution Buffer (AVE) to Lysis Buffer (AL) according to instructions on page 27 (for the manual procedure only).
- If possible, use fresh Elution Buffer (AVE) for each procedure (4 tubes are provided).
- 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.

Procedure

- For the manual procedure with a microcentrifuge, follow steps 1–15.
- This procedure can be automated on the QIAcube Connect MDx in two different versions:
  - Plasma or Serum_Standard: Fully automated using 200 µl of sample (automation starting from step 1)
  - Plasma or Serum_Manual lysis: Partly automated with off-board manual lysis using 200 µl volume of initial sample (automation starting after step 5)

1. Pipet 25 µl QIAGEN Protease (QP) into a lysis tube (LT).

   ⓘ Check the expiration date of the reconstituted protease before use.

2. Add 200 µl of plasma or serum into the lysis tube (LT).

   Note: If the sample volume is less than 200 µl, add the appropriate volume of 0.9% sodium chloride solution to bring the volume of protease and sample up to a total of 225 µl.

3. Add 200 µl Lysis Buffer (AL) (containing 28 µg/ml of carrier RNA and optionally internal control). Close the cap and mix by pulse-vortexing for ≥15 s.

   To ensure efficient lysis, it is essential that the sample and Lysis Buffer (AL) are mixed thoroughly to yield a homogenous 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 min in a heating block.
5. Briefly centrifuge the lysis tube (LT) to remove drops from the inside of the lid.

   Note: If manual lysis (steps 1–15) was done off-board, the following steps (steps 6–15) can be automated: "Manual lysis protocol" on the QIAcube Connect MDx.
6. Add 250 µl ethanol (96–100%) to the sample, close the lid, and mix thoroughly by pulse-vortexing for ≥15 s. Incubate the lysate with the ethanol for 5 min at room temperature (15–25°C).
7. Briefly centrifuge the tube to remove drops from the inside of the lid.
8. Carefully apply all of the lysate from step 7 onto the QIAamp MinElute column without wetting the rim. Close the cap and centrifuge at approximately 6000 *x g* for >1 min. Place the QIAamp MinElute column in a clean 2 ml wash tube (WT), and discard the wash tube (WT) containing the filtrate.

   ⓘ If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the QIAamp MinElute column is empty.
9. Carefully open the QIAamp MinElute column, and add 500 µl Wash Buffer 1 (AW1) without wetting the rim. Close the cap and centrifuge at approximately 6000 *x g* for ≥1 min. Place the QIAamp MinElute column in a clean 2 ml wash tube (WT), and discard the wash tube (WT) containing the filtrate.
10. Carefully open the QIAamp MinElute column, and add 500 µl Wash Buffer 2 (AW2) without wetting the rim. Close the cap and centrifuge at approximately 6000 *x g* for >1 min. Place the QIAamp MinElute column in a clean 2 ml wash tube (WT), and discard the wash tube (WT) containing the filtrate.

11. Carefully open the QIAamp MinElute column and add 500 µl ethanol (96–100%) without wetting the rim. Close the cap and centrifuge at approximately 6000 *x g* for >1 min. Discard the wash tube (WT) containing the filtrate.

    ⓘ Ethanol carryover into the eluate may cause problems in downstream applications. Some centrifuge rotors may vibrate upon deceleration, resulting in the flow-through, which contains ethanol, contacting the QIAamp MinElute column. Removing the QIAamp MinElute column and wash tube (WT) from the rotor may also cause flow-through to come into contact with the QIAamp MinElute column.

12. Place the QIAamp MinElute column in a clean 2 ml wash tube (WT). Centrifuge at full speed (approximately 20,000 *x g*) for 3 min to dry the membrane completely.

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

13. Place the QIAamp MinElute column into a new 2 ml wash tube (WT), open the lid, and incubate the assembly at 56°C for 3 min to dry the membrane completely to evaporate any remaining liquid.

14. Place the QIAamp MinElute column in a new elution tube (ET), and discard the wash tube (WT) with the filtrate. Carefully open the lid of the QIAamp MinElute column, and apply 20–150 µl Elution Buffer (AVE) 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. In the automated workflow elution volumes of 60-100 µl in 5 µl increments are possible. 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.

15. Close the lid and incubate at room temperature for ≥3 min. Centrifuge at full speed (approximately 20,000 *x g*) for 1 min.

ⓘ 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).

ⓘ In case of all automated procedures, remove the eluates from the instrument directly after the finished run and store them properly.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of QIAamp DSP Virus Spin 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 studies.

To minimize the risk of a negative impact on the diagnostic result, 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 lysate has not completely passed through the membrane after centrifugation at 6000 *x g* (8000 rpm), centrifuge again at full speed (up to 20,800 *x g*) for 1 min. If the lysate still does not pass through the membrane during centrifugation, discard the sample and repeat the isolation and purification with new sample material beginning at step 1. 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*. 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.

		Comments and suggestions
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)	For problems in the automated workflow	Refer to *QIAcube Connect MDx User Manual*.

DNA does not perform well in downstream applications

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 Elution 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 Spin 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 Elution 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
	Consult instructions for use
	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
	Important note
	Lot number
	Material number (i.e., component labeling)
	Components
	Volume
	Temperature limitation
	Manufacturer

Symbol	Symbol definition
	Upon arrival
	Open on delivery; store QIAamp MinElute columns at 2–8°C
	Write down the current date after adding ethanol to the bottle
ADD	Adding
CONT	Contains
LYOPH	Lyophilized
RCNS	Reconstitute in
EtOH	Ethanol
GuHCl	Guanidine hydrochloride
MALEIC ACID	Maleic acid
SUBT	Subtilisin
GTIN	Global Trade Item Number
	Leads to
NUM	Number
Rn	R is for revision of the Instructions for Use and n is the revision number
	Keep away from sunlight

Symbol	Symbol definition
	Warning/caution
	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 Spin Kit (50)	For 50 preps: QIAamp MinElute columns, Buffers, Reagents, Tubes, VacConnectors	61704
Related products		
QIAcube Connect MDx*	Instrument and 1-year warranty on parts and labor	9003070
Accessories		
Rotor Adapters	For 240 preps: 240 Disposable Rotor Adapters and 240 Elution Tubes (1.5 ml); for use with the QIAcube Connect MDx	990394
Rotor Adapter Holder	Holder for 12 disposable rotor adapters; for use with the QIAcube Connect MDx	990392
Sample Tubes CB	1000 conical screw-cap tubes without skirted base (2 ml) for use with the QIAcube Connect MDx	990382
Shaker Rack Plugs	For loading the QIAcube Connect MDx shaker rack	9017854
Reagent Bottles, 30 ml	Reagent Bottles (30 ml) with lids; pack of 6; for use with the QIAcube Connect MDx	990393
Filter-Tips, 1000 µl	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube Connect MDx	990352

Filter-Tips, 1000 µl, wide-bore	Disposable Filter-Tips, wide-bore, racked; (8 x 128); not required for all protocols. For use with the QIAcube Connect MDx	990452
Filter-Tips, 200 µl	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube Connect MDx and the QIAsymphony SP/AS instruments	990332

* The QIAcube Connect MDx is not available in all countries. For further details, please contact QIAGEN Technical Service.

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 of 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 ● Update of Appendix Section ● Addition of Troubleshooting Guide ● Update of Symbols section ● Update of Ordering Information

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