January 2021

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

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Version 1

IVD

For In Vitro Diagnostic Use

CE

REF 61704

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R7 MAT 1122785

Sample to Insight

QIAGEN

Contents

Intended Use

The QIAamp DSP Virus Spin Kit is a system that uses silica-membrane technology (QIAamp technology) for isolation and purification of viral nucleic acids from biological specimens.

The product is intended to be used by professional users, such as technicians and physicians that are trained in molecular biological techniques.

The QIAamp DSP Virus Spin Kit is intended for in vitro diagnostic use.

Description and Procedure

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 and 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.

Automated viral nucleic acid purification on the QIAcube or QIAcube Connect MDx

The QIAcube and QIAcube Connect MDx perform 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 or 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.

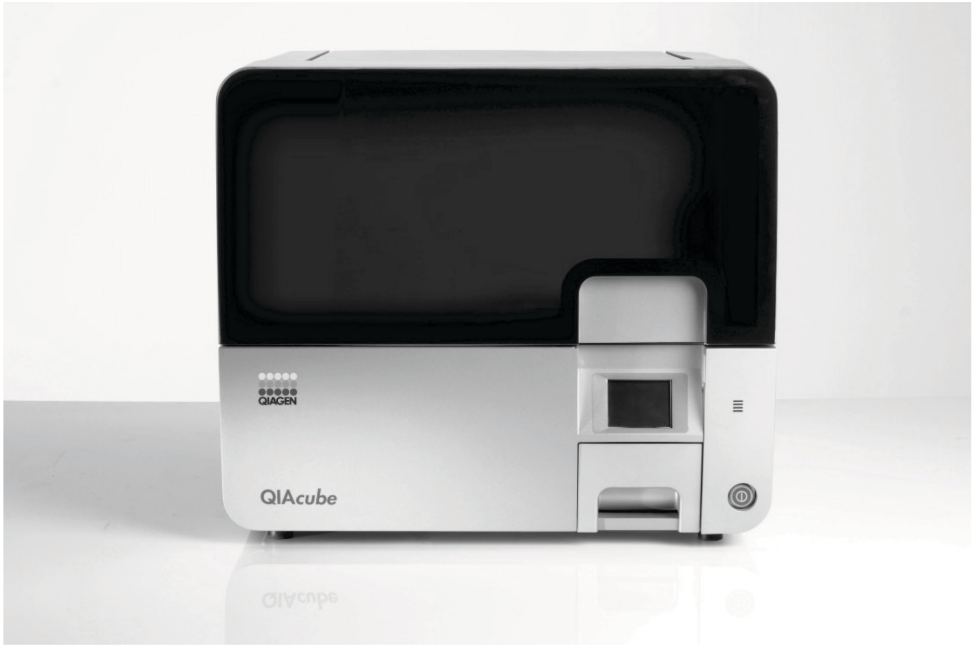


Figure 1. The QIAcube.

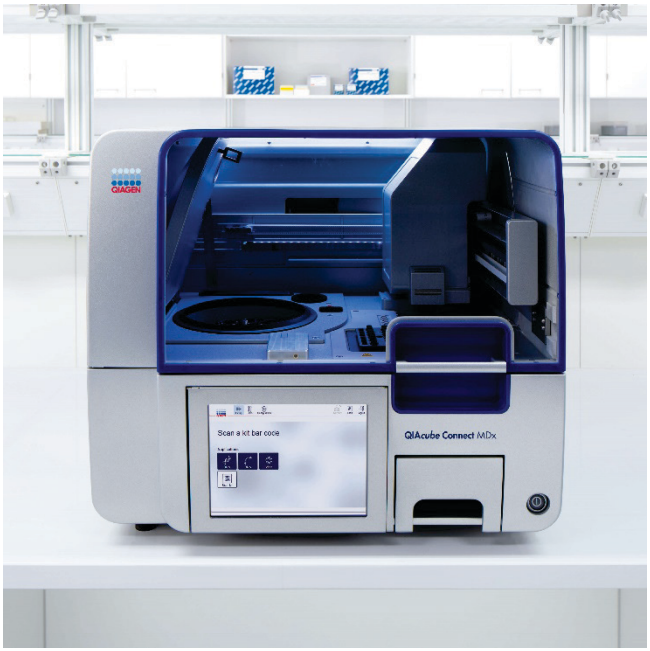


Figure 2. The QIAcube Connect MDx.

Lysis with QIAGEN Protease

Samples are lysed under highly denaturing conditions at elevated temperatures. Lysis is performed in the presence of QIAGEN Protease and 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 tubes (provided) support 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. In a single step, highly pure viral RNA and DNA are eluted in Buffer AVE, equilibrated to room temperature.

Elution of pure nucleic acids

Elution is performed using Buffer AVE. QIAamp MinElute columns allow minimal elution volumes of only 20 µl. Low elution volume leads to highly concentrated nucleic acid eluates.

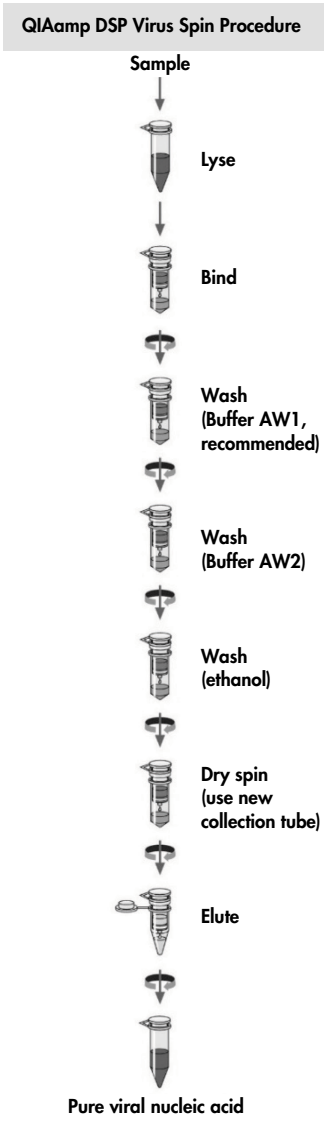
For downstream applications that require small starting volumes (e.g., some PCR and RT-PCR assays), a more concentrated eluate may increase assay sensitivity.

For downstream applications that require a larger starting volume, the elution volume can be increased up to 150 µl. However, an increase in elution volume will decrease the concentration of nucleic acids in the eluate.

The eluate volume recovered can be up to 5 µl less than the volume of elution buffer applied to the column; for example, an elution buffer volume of 20 µl results in >15 µl final eluate. The volume of eluate recovered depends on the nature of the sample.

Eluted nucleic acid is collected in 1.5 ml elution tubes (ET, provided). Storage of DNA or RNA at –30 to –15°C is recommended.

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.



Automatable on the QIAcube/QIAcube Connect MDx

Carrier RNA

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

Different amplification systems vary in efficiency depending on the total amount of nucleic acid 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 be based 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 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.

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. The procedure is suitable for use with plasma and serum. Samples can be either fresh or frozen, provided that they have not been frozen and thawed more than once (see page 19). Viral nucleic acids are eluted in Buffer AVE, ready for use in amplification reactions or storage at –30 to –15°C.

Materials Provided

Kit contents

QIAamp DSP Virus Spin Kit			
Catalog no.			**61704**
Number of preps			**50**[§]
QIAamp MinElute	QIAamp MinElute Columns with Wash Tubes (WT) (2 ml)	COL	50
LT	Lysis Tubes (2 ml)	LYS TUBE	50
ET	Elution Tubes (1.5 ml)	ELU TUBE	50
WT	Wash Tubes (2 ml)	WASH TUBE	5 x 50
AL	Lysis Buffer*	LYS BUF	33 ml
AW1	Wash Buffer 1* (concentrate)	WASH BUF 1 CONC	19 ml
AW2	Wash Buffer 2[†] (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[‡]	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 22.

[§] If automating the QIAamp DSP Virus Spin Kit on the QIAcube or 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.

Materials Required but Not Provided

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

- Ethanol (96–100%)*
- Pipettes† and pipette tips (to prevent cross-contamination, we strongly recommend the use of pipette tips with aerosol barriers)
- Heating block† for lysis of samples at 56°C
- Microcentrifuge† (with rotor for 1.5 ml and 2 ml tubes)
- Vortexer
- For samples <200 µl: 0.9% NaCl solution

For the automated procedure only

- 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
- 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)

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.
† To ensure that samples are properly processed in the QIAamp DSP Virus Spin Kit procedures, we strongly recommend that instruments (e.g., pipets and heating blocks) are calibrated according to the manufacturers' recommendations.

Warnings and Precautions

Please be aware that you may be required to report serious incidents that have occurred in relation to the device to the manufacturer and the regulatory authority in which the user and/or the patient is established.

Safety information

For In Vitro Diagnostic Use

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.

Buffer AL and Buffer 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.

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

Buffer AL

Contains: guanidine hydrochloride; maleic acid. Warning! May be harmful if swallowed or if inhaled. Causes skin irritation. Causes serious eye irritation. May cause an allergic skin reaction. If eye irritation persists: Get medical advice/attention. Take off contaminated clothing and wash it before reuse. Wear protective gloves/protective clothing/eye protection/face protection.

Buffer AW1

Contains: guanidine hydrochloride. Warning! Harmful if swallowed or if inhaled. Causes skin irritation. Causes serious eye irritation. Call a POISON CENTER or doctor/physician if you feel unwell. Dispose of contents/container to an approved waste disposal plant. Take off contaminated clothing and wash it before reuse. Wear protective gloves/protective clothing/eye protection/face protection.

QIAGEN Protease

Contains: Subtilisin. Danger! Causes mild skin irritation. Causes serious eye damage. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/mist/vapors/spray. Dispose of contents/container to an approved waste disposal plant. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. Immediately call a POISON CENTER or doctor/physician. Wear protective gloves/protective clothing/eye protection/face protection. Wear respiratory protection.

Reagent Storage and Handling

QIAamp MinElute columns should be stored at 2–8°C upon arrival. All buffers can be stored at room temperature (15–25°C).

Lyophilized carrier RNA can be stored at room temperature until the expiration date on the kit box. Carrier RNA can only be dissolved in Buffer AVE; dissolved carrier RNA should be immediately added to Buffer AL as described on page 22 for the manual procedure 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 Buffer AVE should be frozen in aliquots at –30 to –15°C.

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

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 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 Storage and Handling

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 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 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.

Procedure

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.
- All centrifugation steps are carried out at room temperature (15–25°C).
- Always use disposable gloves and regularly check that they are not contaminated with sample material. Discard gloves if they become contaminated.
- To minimize cross-contamination, open only one tube at a time.
- 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 ensure safety from potentially infectious material, we recommend working under laminar airflow conditions until the samples are lysed.
- For automation, follow the instructions from the protocol sheets (QIAcube) or on the software screen (QIAcube Connect MDx) and refer to the appropriate user manuals (for the QIAcube and the 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.
- 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.
- After all pulse-vortexing steps, briefly centrifuge the microcentrifuge tubes to remove drops from the inside of the lid.
- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

Centrifugation

- Wash tubes 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 from the microcentrifuge.
- Place the QIAamp MinElute column in a new wash tube. Discard the filtrate and the wash tube. 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 tubes so that the QIAamp MinElute columns can be transferred after centrifugation. Used wash tubes containing the filtrate can be discarded, and the new wash tubes 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 34 before starting.

- Preparing QIAGEN Protease

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

ⓘ Do not add QIAGEN Protease (QP) directly to 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.

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

- Adding carrier RNA to Buffer AL* (for the manual procedure only)

  Add 310 µl 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 –25 to –15°C. Do not freeze–thaw the aliquots of carrier RNA more than 3 times.

  Carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AVE and then added to Buffer AL.

  Calculate the volume of Buffer AL–carrier RNA mix needed per batch of samples by selecting the number of samples to be simultaneously processed from Table 1, page 24. For larger numbers of samples, volumes can be calculated using the sample calculation, below:

  n x 0.22 ml = y ml

  y ml x 28 µl/ml = z µl

  where: n = number of samples to be processed simultaneously

  y = calculated volume of Buffer AL

  z = volume of carrier RNA–Buffer AVE to add to Buffer AL

Gently mix by inverting the tube 10 times. To avoid foaming, do not vortex. For the automated procedure, the addition of carrier RNA to Buffer AL is done by the QIAcube/QIAcube Connect MDx.

* 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.

Table 1. Volumes (Vol.) of Buffer AL and carrier RNA–Buffer AVE mix required for specific numbers (No.) of samples for the QIAamp DSP Virus Spin procedure

No. samples	Vol. Buffer AL (ml)	Vol. carrier RNA AVE (µl)	No. samples	Vol. 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 Buffer AL. For each microgram of carrier RNA required per preparation, add 5 µl Buffer AVE-dissolved carrier RNA per milliliter of Buffer AL. Use of less than 5.6 µg carrier RNA per sample must be validated for each particular sample type and downstream assay.

Buffer AW1 *

Add 25 ml of ethanol (96–100%) to a bottle containing 19 ml of Buffer AW1 concentrate, as described on the bottle. Tick the check box on the label to indicate that ethanol has been added. Store reconstituted Buffer AW1 at room temperature. Reconstituted Buffer AW1 is stable for up to one year when stored at room temperature, but only until the kit expiration date.

Always mix reconstituted Buffer AW1 by shaking before starting the procedure.

Buffer AW2 †

Add 30 ml of ethanol (96–100%) to a bottle containing 13 ml of Buffer AW2 concentrate, as described on the bottle. Tick the check box on the label to indicate that ethanol has been added. Store reconstituted Buffer AW2 at room temperature. Reconstituted Buffer AW2 is stable for up to one year when stored at room temperature, but only until the kit expiration date.

Always mix reconstituted Buffer AW2 by shaking before starting the procedure.

Elution of nucleic acids

Elution buffer should be equilibrated to room temperature before it is applied to the column.

* 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/QIAcube Connect MDx

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

Important points before starting

- All centrifugation steps are carried out at room temperature (15–25°C).
- 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 or QIAcube Connect MDx.
- For automation, follow the instructions from the Protocol Sheets (QIAcube) or on the software screen (QIAcube Connect MDx) and refer to the appropriate user manuals (for the QIAcube and the QIAcube Connect MDx).

Things to do before starting

- Equilibrate samples to room temperature (15–25°C).
- Equilibrate Buffer AVE to room temperature for elution in step 14.
- Set a heating block to 56°C ± 3°C for use in step 4.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease (QP) have been prepared according to instructions on pages 20–25.
- Add carrier RNA reconstituted in Buffer AVE to Buffer AL according to instructions on page 22 (for the manual procedure only).

Procedure

- For the manual procedure with a microcentrifuge, follow steps 1–14
- This procedure can be automated on the QIAcube Connect MDx in two different versions:
  - Plasma or Serum_Standard: Full automation using 200 µl of sample (starting from step 1)
  - Plasma or Serum_Manual lysis: Partly automated with off-board manual lysis using 200 µl volume of initial sample (starting after step 5)

  Note: For protocol selection on the QIAcube, please refer to the protocol sheets (**https://www.qiagen.com/us/qiacube/standard/search/**).

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

Read "Preparing reagents and buffers", page 22, for information about resuspending QIAGEN Protease (QP) in Protease Solvent (PS).

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

   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 Buffer AL (containing 28 µg/ml of carrier RNA). Close the cap and mix by pulse-vortexing for ≥15 s.

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

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

4. Incubate at 56°C ± 3°C for 15 min ± 1 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 1–5) was done off-board, the following steps (steps 6–14) can be automated: "Manual lysis protocol" on the QIAcube or QIAcube Connect MDx or "Large Plasma samples_Manual lysis protocol" on the QIAcube.

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 ± 30 s at room temperature (15–25°C).

ⓘ If ambient temperature exceeds 25°C, ethanol should be cooled on ice before adding to the lysate.

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 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 Buffer 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 containing the filtrate.
10. Carefully open the QIAamp MinElute column, and add 500 µl Buffer 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, and discard the wash tube 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 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 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 ± 30 s to dry the membrane completely.
13. Place the QIAamp MinElute column into a new 2 ml wash tube (WT), open the lid, and incubate the assembly at 56°C ± 3°C for 3 min ± 30 s to dry the membrane completely.

    This step serves to evaporate any remaining liquid.
14. Place the QIAamp MinElute column in an elution tube (ET), and discard the wash tube with the filtrate. Carefully open the lid of the QIAamp MinElute column, and apply 20–150 µl Buffer AVE to the center of the membrane. Close the lid and incubate at room temperature for 5 min. Centrifuge at full speed (approximately 20,000 x *g*) for >1 min.

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

ⓘ Ensure that the elution buffer is equilibrated to room temperature. If elution is done in small volumes (<50 µl), the elution buffer must be dispensed onto the center of the membrane for complete elution of bound RNA and DNA.

Elution volume is flexible and can be adapted according to the requirements of the downstream application. Remember that the recovered eluate volume will be approximately 5 µl less than the elution buffer volume applied onto the column.

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 using plasma and serum samples for isolation of viral nucleic acids.

It is the user's responsibility to validate 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 results, adequate controls for downstream applications should be used. For further validation, the guidelines of the International Conference on Harmonization of Technical Requirements (ICH) in *ICH Q2(R1) Validation Of Analytical Procedures: Text And Methodology* are recommended.

Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

Symbols

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

Symbol	Symbol definition
	Contains reagents sufficient for <N> reactions
	Consult instructions for use
	Use by
	In vitro diagnostic medical device
	Catalog number
	Important note
	Lot number
	Material number (i.e., component labeling)
	Components
	Volume
	Temperature limitation
	Manufacturer
	Upon arrival

Symbol	Symbol definition
	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
	Warning/caution

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support** (for contact information, visit **www.qiagen.com**).

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.

Nondisposable plasticware

Nondisposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH,* 1 mM EDTA* followed by RNase-free water* (see "Solutions", page 35). Alternatively, chloroform-resistant plasticware can be rinsed with chloroform* to inactivate RNases.

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

Glassware

Glassware should be treated before use to ensure that it is RNase-free. Glassware used for RNA work should be cleaned with detergent, thoroughly rinsed and oven-baked at >240°C for four or more hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Oven baking will both inactivate ribonucleases and ensure that no other nucleic acids (such as plasmid DNA) remain on the surface of the glassware. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Cover the glassware with 0.1% DEPC in water overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to remove residual DEPC.

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

Electrophoresis tanks

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

Solutions

Solutions (water and other solutions) should be treated with 0.1% DEPC. DEPC will react with primary amines and cannot be used directly to treat Tris buffers. DEPC is highly unstable in the presence of Tris buffers and decomposes rapidly into ethanol and CO_2. When preparing Tris buffers, treat water with DEPC first, and then dissolve Tris to make the appropriate buffer.

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

† Contains sodium azide as a preservative.

DEPC is a strong, but not absolute, inhibitor of RNases. It is commonly used at a concentration of 0.1% to inactivate RNases on glass or plasticware or to create RNase-free solutions and water. DEPC inactivates RNases by covalent modification. Trace amounts of DEPC will modify purine residues in RNA by carbethoxylation. Carbethoxylated RNA is translated with very low efficiency in cell-free systems. However, its ability to form DNA:RNA or RNA:RNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be removed from solutions or vessels by autoclaving or heating to 100°C ± 3°C for 15 minutes ± 1 minute.

Add 0.1 ml DEPC to 100 ml of the solution to be treated, and shake vigorously to bring the DEPC into solution or let the solution incubate for >12 hours at 37°C ± 3°C. Autoclave for 15 minutes ± 1 minute to remove any trace of DEPC. It may be desirable to test water sources for the presence of contaminating RNases since many sources of distilled water are free of RNase activity.

ⓘ QIAamp DSP Virus Spin Kit buffers are not rendered RNase-free by DEPC treatment and are therefore free of any DEPC contamination.

Ordering Information

Product	Contents	Cat. no.
QIAamp DSP Virus Spin Kit (50)	For 50 preps: QIAamp Mini Spin 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	990394
Rotor Adapter Holder	Holder for 12 disposable rotor adapters; for use with the QIAcube	990392
Sample Tubes CB	1000 conical screw-cap tubes without skirted base (2 ml) for use with the QIAcube and QIAcube Connect	990382
Shaker Rack Plugs	For loading the QIAcube shaker rack	9017854
Reagent Bottles, 30 ml	Reagent Bottles (30 ml) with lids; pack of 6; for use with the QIAcube	990393
Filter-Tips, 1000 µl	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube	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	990452

Product	Contents	Cat. no.
Filter-Tips, 200 µl	Disposable Filter-Tips, racked; (8 x 128). For use with the QIAcube 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 handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

Document Revision History

Revision	Description
R7, 01/2021	Updated the following sections: "Automated viral nucleic acid purification on the QIAcube or QIAcube Connect MDx", "Materials Required but Not Provided", "Warnings and Precautions", "Protocol: Purification of viral nucleic acids from plasma or serum using a microcentrifuge or the QIAcube/QIAcube Connect MDx", "Symbols", and "Ordering Information" sections. Removed the “Performance Characteristics” and "References" sections. Inserted a new figure (image of the QIAcube Connect MDx). Added references to the QIAcube Connect MDx and its accessories. Editorial and layout changes.

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