June 2015

MagAttract® Virus Mini M48 Handbook

For simultaneous purification of viral DNA and RNA from serum, plasma, or cell-free body fluids using the BioRobot® M48 workstation

For use with
MagAttract Virus Mini protocol v1.4
MagAttract Virus IC protocol v2.2

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QIAGEN Sample and Assay Technologies

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Contents

Kit Contents

MagAttract Virus Mini M48 Kit	**(192)**
Catalog no.	**955336**
Number of preps	**192**
MagAttract Suspension B	11 ml
QIAGEN® Protease*	4 vials
Protease Resuspension Buffer†	4 x 6 ml
Buffer AL‡	4 x 33 ml
Buffer AW1‡ (concentrate)	2 x 27 ml
Buffer AW2† (concentrate)	66 ml
Buffer AVE†	4 x 125 ml
Carrier RNA	1350 µg
Quick-Start Protocol	1

* Resuspension volume 5.5 ml per vial.

† Contains sodium azide as a preservative.

‡ Contains a guanidine salt. Not compatible with disinfectants containing bleach. See page 5 for safety information.

Shipping and Storage

The MagAttract Virus Mini M48 Kit is shipped at room temperature (15–25°C). All buffers and reagents can be stored at room temperature. Do not freeze MagAttract Suspension B.

Lyophilized carrier RNA is stable for up to 1 year when stored at room temperature. Carrier RNA may only be dissolved in Buffer AVE or an internal control (if used). Dissolved carrier RNA should be immediately frozen in aliquots at –30 to –15°C or added to Buffer AL as described in "Dissolving carrier RNA and adding to Buffer AL" on page 10. Buffer AL containing carrier RNA should be prepared fresh, and is stable at 2–8°C for up to 48 hours.

Lyophilized QIAGEN Protease can be stored at room temperature for up to 1 year. For longer storage, or if ambient temperatures exceed 25°C, lyophilized QIAGEN Protease should be stored dry at 2–8°C.

Reconstituted QIAGEN Protease is stable for 1 year when stored at 2–8°C. Avoid storage at room temperature for prolonged periods. For longer storage, we recommend freezing aliquots at –30 to –15°C and avoiding repeated freezing and thawing.

Intended Use

The MagAttract Virus Mini M48 Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of MagAttract Virus Mini M48 Kits is tested against predetermined specifications to ensure consistent product quality.

Introduction

The MagAttract Virus Mini M48 Kit provides reagents for 2 fully automated procedures for simultaneous purification of viral DNA and RNA from serum and plasma, the MagAttract Virus Mini protocol v1.4 and the MagAttract Virus IC protocol v2.2. The kit can be used to purify nucleic acids from a broad range of DNA and RNA viruses. However, kit performance is not guaranteed for each virus species and has to be validated by the user. MagAttract technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. The purified nucleic acids are ready for direct use in downstream applications, such as amplification or other enzymatic reactions. The BioRobot M48 workstation performs all steps of the purification procedure. Up to 48 samples, in multiples of 6, are processed in a single run.

Description of protocols

The **MagAttract Virus Mini protocol v1.4** processes 400 µl of sample input volume. Purification time is less than 5 minutes per sample (when 48 samples are processed in parallel). The protocol allows addition of internal control by making a premix of internal control and lysis Buffer AL, as described in "Adding internal control", page 12.

The **MagAttract Virus IC protocol v2.2** is flexible in processing sample input volumes of 50, 100, 200, or 400 µl. Purification time is approximately 6 minutes per sample (when 48 samples are processed in parallel). The protocol offers 2 ways to introduce internal controls into the sample preparation: packaged internal controls, which require lysis (e.g., armored RNA, etc.), should be added with the lysis buffer as described in "Adding internal control", page 12. Native internal controls, which do not require lysis (e.g., in vitro transcripts, plasmids, etc.), can be added automatically during the extraction process at the binding step, as described in "Adding internal control", page 12. This protocol uses a milder lysis procedure, an optimized binding procedure, and maximizes removal of wash buffer carryover into the eluate. Thus, the overall performance of purified viral nucleic acids is significantly improved in comparison to the MagAttract Virus Mini protocol v1.4.

Principle and procedure

MagAttract technology combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles. The purification procedure is designed to ensure safe and reproducible handling of potentially infectious samples. The purification procedure comprises 4 steps: lyse, bind, wash, and elute (see next page, and flowchart, page 8).

Lysis with QIAGEN Protease

Proteolysis of cell-free body fluids, serum, or plasma samples is carried out under highly denaturing conditions. Lysis is performed in the presence of QIAGEN Protease and Buffer AL, which together ensure digestion of viral coat proteins and inactivation of RNases.

Binding to MagAttract magnetic particles

Isopropanol is added to the lysed samples to adjust binding conditions. Lysates are thoroughly mixed with MagAttract Suspension B to allow optimal adsorption of viral DNA and RNA to the silica surface of the magnetic particles. Salt and pH conditions ensure that protein and other contaminants, which can inhibit PCR and other downstream enzymatic reactions, are not bound to the magnetic particles.

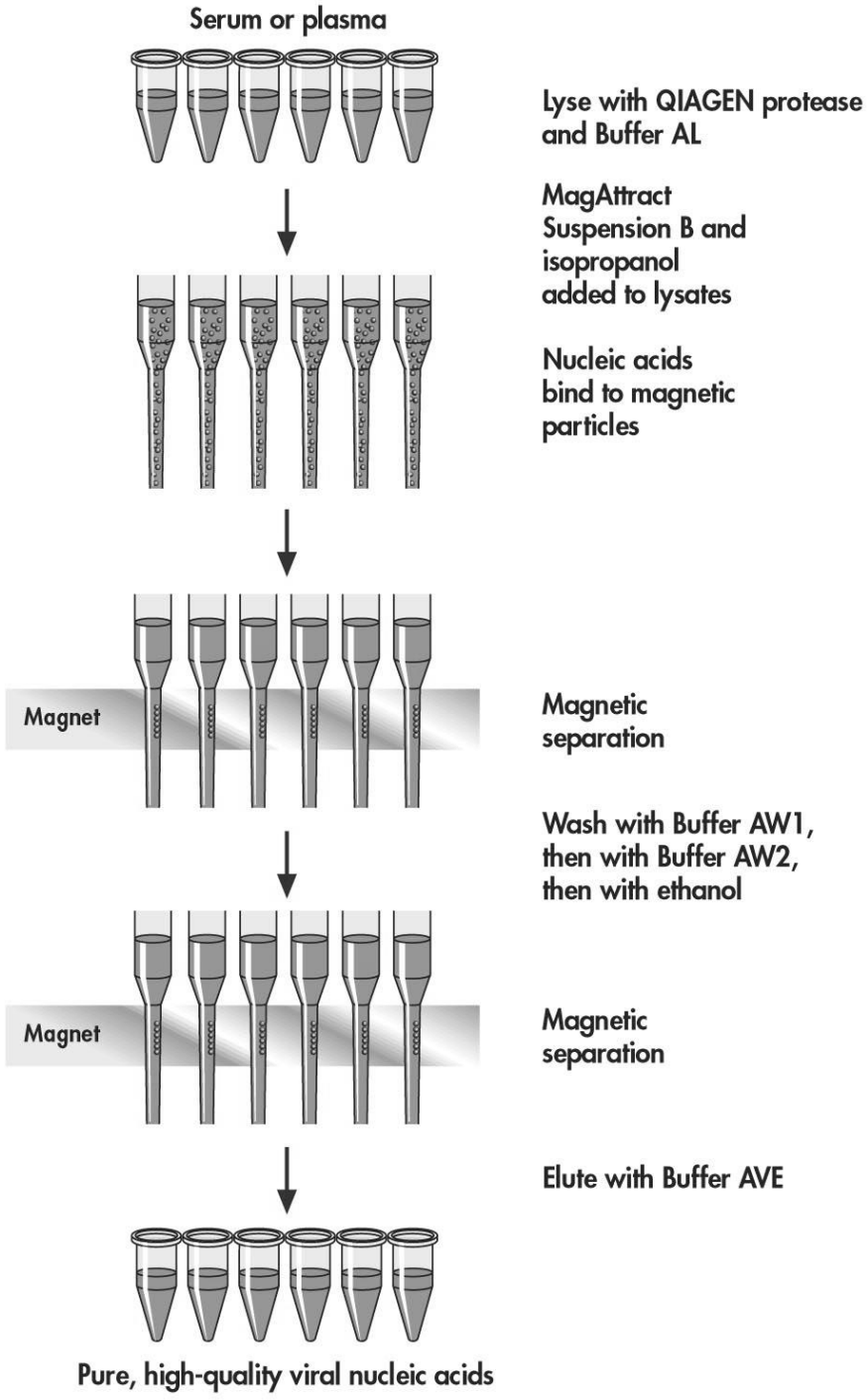
Washing of bound nucleic acids

While viral DNA and RNA remain bound to the magnetic particles, contaminants are efficiently washed away during a sequence of wash steps using first Buffer AW1, then Buffer AW2, and then ethanol.

Elution of pure nucleic acids

In a single step, highly pure viral DNA and RNA are eluted in Buffer AVE equilibrated to room temperature (15–25°C). Buffer AVE is RNase-free water with 0.04% sodium azide to prevent microbial growth and subsequent contamination with RNases. Sodium azide affects spectrophotometric absorbance readings between 220 and 280 nm but has no effect on downstream applications, such as RT-PCR. The purified nucleic acids can be either used immediately in downstream applications or stored for future use.

MagAttract Virus Mini M48 Procedure



Equipment and Reagents to Be Supplied by User

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

- BioRobot M48 workstation
- App. Package, M48, Inf. Dis., v3.2, cat. no. 9016145
- Filter-Tips, 1000 µl, M48 (1000), cat. no. 995652
- Reagent Containers, small, M48 (100), cat. no. 995902
- Reagent Containers, large, M48 (50), cat. no. 995904
- Optional: Reagent Container Seals, M48 (50), cat. no. 995906
- Sample Prep Plates, 42-well, M48 (100), cat. no. 995908
- Sample tubes, 2 ml, without lids (Sarstedt, cat. no. 72.608)* or with screw caps (Sarstedt, cat. no. 72.693)*
- Microcentrifuge tubes for elution, 1.5 ml, with screw caps (Sarstedt, cat. no. 72.692)*
- Sterile, RNase-free pipet tips
- Disposable gloves
- Ethanol (96–100%)†
- Isopropanol

* This is not a complete list of suppliers and does not include many important vendors of biological supplies; however, use of other tubes may result in an instrument crash.

† Do not use denatured ethanol, which contains other substances such as methanol or methylethylketone.

Important Notes

Preparing serum and plasma samples

The purification procedure is optimized for use with cell-free body fluids, serum, or plasma 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.

After collection and centrifugation, plasma or serum can be stored at 2–8°C for up to 6 hours. For longer storage, we recommend freezing aliquots at –15 to –30°C or –80°C. Frozen plasma or serum must not be thawed more than once. Repeated freeze–thawing leads to denaturation and precipitation of proteins, resulting in a potential reduction in viral titers and therefore reduced yields of viral nucleic acids. If cryoprecipitates are visible in the samples, centrifuge at 6800 x *g* for 3 minutes, transfer the supernatants to fresh tubes without disturbing the pellets, and start the purification procedure immediately. Centrifugation at low *g*-forces does not reduce viral titers.

Reconstituting QIAGEN Protease

Add 5.5 ml Buffer AVE to a vial of lyophilized QIAGEN Protease and mix carefully to avoid foaming. Make sure that the QIAGEN Protease is completely dissolved. Store the reconstituted QIAGEN Protease at 2–8°C. We recommend freezing aliquots at –15 to –30°C. Make sure the Buffer AVE bottle does not become contaminated with QIAGEN Protease.

Note: For some starting materials, such as EDTA plasma, dissolving lyophilized QIAGEN Protease in Protease Resuspension Buffer may increase lysis efficiency. However, Protease Resuspension Buffer is not compatible with samples or internal controls containing phosphate buffer (e.g., viral transport medium, PBS). If the sample or internal control contains phosphate buffer, it is highly recommended to resuspend QIAGEN Protease in Buffer AVE (supplied with the kit). Reconstituting QIAGEN Protease in Buffer AVE is also recommended for use with serum samples. The user should evaluate system performance to determine which reconstitution method is best suited for specific samples.

Dissolving carrier RNA and adding to Buffer AL

Carrier RNA is added to Buffer AL and serves 2 purposes. Firstly, it enhances binding of viral nucleic acids to the silica surface of the magnetic particles, especially if the sample contains very few target molecules. Secondly, the addition of large amounts of carrier RNA reduces the chances of viral RNA degradation in the rare event that RNases are not denatured by the chaotropic salts and detergent in Buffer AL. If carrier RNA is not added to Buffer AL, recovery of viral DNA or RNA may be reduced.

The lyophilized carrier RNA provided with the kit is sufficient for the volume of Buffer AL supplied with the kit. The concentration of carrier RNA used in the purification procedure allows the MagAttract Virus Mini M48 Kit to be used as a generic purification system that is compatible with many different amplification systems and suitable for purifying a wide range of DNA and RNA viruses. However, amplification systems vary in efficiency depending on the total amount of nucleic acids present in the reaction. Eluates obtained using this kit contain both viral nucleic acids and carrier RNA, and in each eluate, the amount of carrier RNA greatly exceeds the amount of viral nucleic acids. The amount of eluate to add to downstream amplification reactions should therefore be based on the amount of carrier RNA in the eluate. To obtain the highest levels of sensitivity in amplification reactions, it may be necessary to adjust the amount of carrier RNA solution added to Buffer AL.

Add 1350 µl Buffer AVE to the tube containing 1350 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 at –15 to –30°C. Do not freeze–thaw the aliquots more than 3 times. Note that carrier RNA does not dissolve in Buffer AL. It must first be dissolved in Buffer AVE and then added to Buffer AL.

The BioRobot M48 calculates the volumes of Buffer AL and carrier RNA–Buffer AVE required for a protocol run after the user has entered into the QIAsoft M software the number of samples to be processed. Alternatively, refer to Table 1 below for the volumes of carrier RNA solution and Buffer AL to use.

Table 1. Volumes of Buffer AL and carrier RNA–Buffer AVE required for the MagAttract Virus Mini procedure

Number of samples	Volume of Buffer AL (ml)	Volume of carrier RNA–Buffer AVE (µl)
6	3.8	26
12	6.4	44
18	9.0	62
24	11.7	81
30	14.3	99
36	16.9	117
42	19.5	134
48	22.1	152

After adding carrier RNA–Buffer AVE mix to Buffer AL, gently mix by inverting the tube 10 times. To avoid foaming, do not vortex.

Note: The purification procedure is optimized so that 3 µg carrier RNA is added 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 2.3 µl Buffer AVE-dissolved carrier RNA per milliliter of Buffer AL. (Use of less than 3 µg carrier RNA per sample must be validated for each particular sample type and downstream assay.)

Adding internal control

Using the MagAttract Virus Mini M48 Kit in combination with commercially available amplification systems may require the introduction of an internal control into the purification procedure to monitor the efficiency of sample preparation and downstream assay. The MagAttract Virus Mini protocol v1.4 and the MagAttract Virus IC protocol v2.2 offer 2 different options to include internal controls.

The MagAttract Virus Mini protocol v1.4 enables addition of internal controls by making a premix of internal control, carrier RNA, and Buffer AL. During the automated purification process, the BioRobot M48 workstation transfers 435 µl of Buffer AL–internal control premix from the small reagent container 3 ("ReaS3") to the lysis reaction.

The MagAttract Virus IC protocol v2.2 allows 2 ways to introduce internal controls into the sample preparation:

i) Internal controls that are packaged into protein coats (e.g., armored RNA) and require lysis should be added to Buffer AL and the carrier RNA as described above.

ii) Native internal controls, which do no require lysis (e.g., in vitro transcripts, plasmids, etc.), can be automatically added during the purification process at the binding step. This option is extremely effective for labile or very short internal control RNA molecules. To use this option, an aliquot of diluted internal control has to be pipetted manually during the worktable set-up into well 6 of the sample preparation plate for each sample processed (Figure 1, page 13). The internal control should be diluted with Buffer AVE containing 10 ng of carrier RNA per microliter. To calculate the correct dilution of internal control, note that 60 µl of diluted internal control has to be pipetted into each well, but the BioRobot M48 workstation will only transfer 30 µl of the solution from well 6 into the binding reaction. The final concentration of the internal control will also depend on the elution volume chosen. When using a commercial assay, refer also to the instructions given by the assay provider.

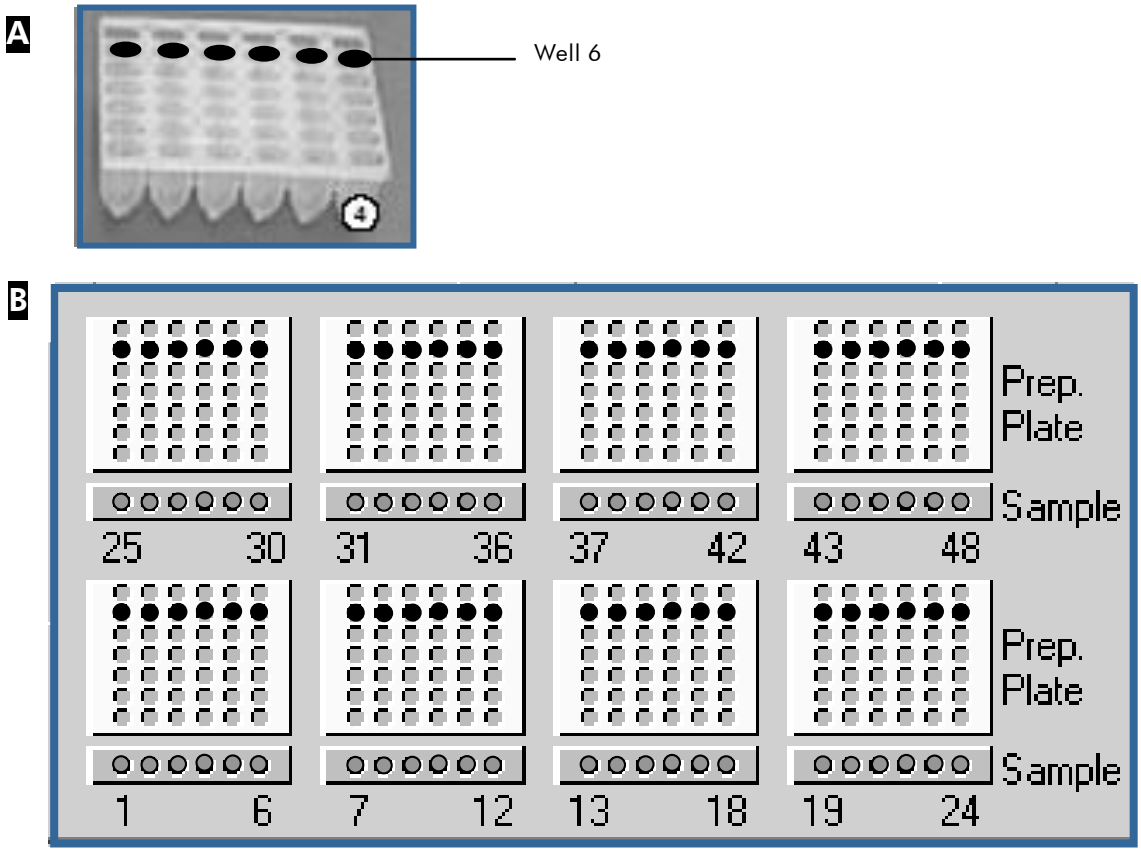


Figure 1. Adding internal control to sample preparation plate. A Sample preparation plate. B Worktable overview for 48 processed samples. Black dots indicate positions where diluted internal control should be added.

Internal control DNA or RNA should be added together with the carrier RNA to Buffer AL or into well 6 of the reaction plate. Dilute with Buffer AVE if necessary. If the internal control is supplied as a lyophilized powder, first dissolve both carrier RNA and internal control in Buffer AVE, and then add to Buffer AL or to the reaction plate.

Refer to the manufacturer's instructions to determine the optimal concentration of internal control. Using a concentration other than that recommended may reduce amplification efficiency. See "Appendix A: Calculating the Amount of Internal Control", page 26, for detailed instructions on how to calculate the correct volume of internal control.

Note: If internal controls are phosphate-buffered (e.g. PBS, viral transport media, some lyophilized standards), QIAGEN Protease must be resuspended in Buffer AVE (see "Reconstituting QIAGEN Protease", page 10). Protease Resuspension Buffer is not compatible with phosphate-buffered starting material.

Preparing Buffer AW1

Add 35 ml ethanol (96–100%) to a bottle containing 27 ml Buffer AW1 concentrate, as described on the bottle. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW1 is stable for 1 year when stored at room temperature (15–25°C).

Note: Always mix reconstituted Buffer AW1 by shaking before starting a purification procedure.

Preparing Buffer AW2

Add 160 ml ethanol (96–100%) to a bottle containing 66 ml Buffer AW2 concentrate, as described on the bottle. Tick the check box on the bottle label to indicate that ethanol has been added. Reconstituted Buffer AW2 is stable for 1 year when stored at room temperature (15–25°C).

Note: Always mix reconstituted Buffer AW2 by shaking before starting a purification procedure.

Handling Buffer AVE

If you have not previously worked with RNA and are purifying viral RNA, read "Appendix B: General Remarks on Handling RNA", page 30, before starting the purification procedure.

Buffer AVE is RNase-free upon delivery. It contains sodium azide, an antimicrobial agent that prevents growth of RNase-producing organisms. However, as this buffer does not contain any RNase-degrading chemicals, it will not actively inhibit RNases introduced by inappropriate handling. When handling Buffer AVE, take extreme care to avoid contamination with RNases.

Yields of viral nucleic acids

The yields of viral nucleic acids obtained in the purification procedure are normally below 1 µg and therefore difficult to quantify using a spectrophotometer. We recommend using quantitative amplification methods to determine yields. Remember that the purified nucleic acids contain much more carrier RNA than viral nucleic acids.

Storing viral nucleic acids

For short-term storage of up to 24 hours, we recommend storing the purified viral DNA and RNA at 2–8°C. For long-term storage of over 24 hours, we recommend storage at –15 to –30°C.

Protocol: Purification of Viral DNA and RNA using the MagAttract Virus IC Protocol v2.2

The MagAttract Virus IC protocol v2.2 is flexible in processing sample input volumes of 50, 100, 200, or 400 µl. The protocol can be used with plasma, serum, or cell-free body fluids. Purification time is approximately 6 minutes per sample with batches of 48 samples per run.

The protocol offers 2 alternative ways to introduce internal controls into the sample preparation:

Packaged internal controls, which require lysis (e.g., armored RNA, etc.), should be added with the lysis buffer as described in "Adding internal control", page 12. During the automated purification process, the BioRobot M48 workstation transfers 435 µl of Buffer AL–internal control premix from the small reagent container 3 ("ReaS3") to the lysis reaction.

Native internal controls, which do not require lysis (e.g., in vitro transcripts, plasmids, etc.), can be added automatically during the extraction process at the binding step, as described in "Adding internal control", page 12. During the automated purification process the BioRobot M48 workstation transfers 30 µl of diluted internal control from well 6 of the reaction plate to the lysis reaction.

This protocol uses a milder lysis procedure, an optimized binding procedure, and maximizes removal of wash buffer carryover into the eluate. Thus, the overall performance is significantly improved in comparison with the MagAttract Virus Mini protocol v1.4.

Things to do before starting

- Install App. Package, M48, Inf. Dis., v3.2 before running this protocol for the first time.
- Ensure that the appropriate cooling block is installed at the "Heat/Cool Block 2" slot of the worktable. For details, refer to the *BioRobot M48 User Manual*.
- Ensure that MagAttract Suspension B is fully resuspended. Vortex for at least 3 min before the first use, and for 1 min before subsequent uses.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to the instructions on pages 10–14.
- Add carrier RNA reconstituted in Buffer AVE to Buffer AL according to the instructions on page 10.
- Dilute internal control with Buffer AVE and carrier RNA according to the instructions in "Dissolving carrier RNA and adding to Buffer AL", page 10 and "Adding internal control", page 12.

Procedure

1. **Switch on the BioRobot M48 workstation.**
   The power switch is on the left side of the workstation.
2. **Switch on the computer and monitor.**
3. **Launch the QIAsoft M Operating System.**
   Upon startup, the computer displays the QIAsoft M startup window. Click "Start" to continue.

   If the QIAsoft M startup window does not appear, either double-click the QIAsoft M icon on the desktop or click the Microsoft® Windows® "Start" menu and select QIAsoft M Operating System → QIAsoft M v2.0* for BioRobot M48.
4. **Click the dark green arrow button and select "Infectious Disease" from the drop-down menu that appears. Then select "Viral NA" and the protocol "Virus IC v2.2", and click the "Select" button.**
5. **In the dialog box that appears, click "1.5 ml" to select 1.5 ml elution tubes.**
   Note: The protocol is designed for elution into 1.5 ml tubes only. Do not click the "0.2 ml" or "2.0 ml" button for selecting 0.2 ml or 2 ml elution tubes.
6. **Select the number of samples, the sample volume of *50*, *100*, *200*, or *400 µl*, and the elution volume of *50*, *75*, *100*, *125*, or *150 µl* in the corresponding dialog fields. Click "Next".**
7. **The QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48 for the protocol selected. The software indicates the volumes of reagents required and the locations on the worktable in which to load the reagents and plasticware. Be sure to follow all instructions that appear. Wear gloves when loading the required items on the worktable.**
   Note: If samples or internal controls are phosphate-buffered (e.g. PBS, viral transport media, some lyophilized standards), QIAGEN Protease must be resuspended in Buffer AVE. Protease Resuspension Buffer is not compatible with phosphate-buffered starting material.

* Or later version.

8. **At the last step of worktable setup, the software prompts you to load your samples onto the worktable. Transfer the requested volume of sample into 2 ml sample tubes and equilibrate to room temperature (15–25°C) before loading on the worktable. If using frozen samples, thaw and equilibrate at room temperature, mix well by vortexing, remove the lids, and immediately load onto the worktable. Click "Next".**

   Note: Avoid freezing and thawing samples more than once or storing samples for over 6 hours at 2–8°C, as this leads to significantly reduced yields of viral nucleic acids.

9. **The software provides you with the option of entering names for your samples.**
10. **Close the workstation door and start the protocol when instructed by the software. All subsequent steps are automated. The software displays a table of results when the protocol is finished.**
11. **Retrieve the elution tubes containing the purified viral nucleic acids from cooling block 1 at the rear of the worktable. The viral nucleic acids are ready to use, or can be stored at 2–8°C for 24 h or at –15 to –30°C to –70°C for longer periods.**

    Note: Some viral RNAs are very rich in secondary structures. A post-elution heating step (e.g., 20 min at 75°C) of open elution tubes leads to significant improvement in performance for certain downstream assays. The post-elution protocol v1.1 on the App. Package, M48, Inf. Dis., v3.2 can be used to perform this heating automatically in the heating/cooling block on the BioRobot M48 worktable. However, for other quantitative downstream assays, this heat incubation has a negative impact. It is recommended to validate the effect of such a post-elution heating step for each individual downstream assay.

Protocol: Purification of Viral DNA and RNA using the MagAttract Virus Mini Protocol v1.4

The MagAttract Virus Mini protocol v1.4 processes 400 µl of sample input volume. Purification time is less than 5 minutes per sample with batches of 48 samples per run. The protocol allows addition of internal control by making a premix of internal control and lysis Buffer AL, as described in "Adding internal control", page 12. During the automated purification process the BioRobot M48 workstation transfers 435 µl of Buffer AL–internal control premix from the small reagent container 3 ("ReaS3") to the lysis reaction. Compared with the MagAttract Virus IC protocol v2.2, this protocol uses a more aggressive lysis procedure and a post-elution heating step. However, overall performance, especially for heat labile RNA viruses, is significantly improved when using the MagAttract Virus IC protocol v2.2, page 15.

Things to do before starting

- Install App. Package, M48, Inf. Dis., v3.2 before running this protocol for the first time.
- Ensure that the appropriate cooling block is installed at the "Heat/Cool Block 2" slot of the worktable. For details, refer to the *BioRobot M48 User Manual*.
- Ensure that MagAttract Suspension B is fully resuspended. Vortex for at least 3 min before the first use, and for 1 min before subsequent uses.
- Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to the instructions on pages 10–14.
- Add carrier RNA reconstituted in Buffer AVE and internal control (if used) to Buffer AL according to the instructions on page 10.

Procedure

1. **Switch on the BioRobot M48 workstation.**
   The power switch is on the left side of the workstation.
2. **Switch on the computer and monitor.**

3. **Launch the QIAsoft M Operating System.**

   Upon startup, the computer displays the QIAsoft M startup window. Click "Start" to continue.

   If the QIAsoft M startup window does not appear, either double-click the QIAsoft M icon on the desktop or click the Microsoft Windows "Start" menu and select QIAsoft M Operating System → QIAsoft M v2.2* for BioRobot M48.

4. **Click the dark green arrow button and select "Infectious Disease" from the drop-down menu that appears. Then select "Viral NA" and the protocol "Virus Mini v1.4" and click the "Select" button.**

5. **In the dialog box that appears, click "1.5 ml" to select 1.5 ml elution tubes.**

   Note: The protocol is designed for elution into 1.5 ml tubes only. Do not click the "0.2 ml" or "2.0 ml" button for selecting 0.2 ml or 2 ml elution tubes.

6. **Select the number of samples, the sample volume of *400 µl* and the elution volume of *50*, *75*, *100*, *125*, or *150 µl* in the corresponding dialog fields. Click "Next".**

7. **The QIAsoft M software will now guide you through the remaining steps required to set up the BioRobot M48 for the protocol selected. The software indicates the volumes of reagents required and the locations on the worktable in which to load the reagents and plasticware. Be sure to follow all instructions that appear. Wear gloves when loading the required items on the worktable.**

   Note: If samples or internal controls are phosphate-buffered (e.g., PBS, viral transport media, some lyophilized standards), QIAGEN Protease must be resuspended in Buffer AVE. Protease Resuspension buffer is not compatible with phosphate-buffered starting material.

8. **At the last step of worktable setup, the software prompts you to load your samples onto the worktable. Transfer the requested volume of sample into 2 ml sample tubes and equilibrate to room temperature (15–25°C) before loading on the worktable. If using frozen samples, thaw and equilibrate at room temperature, mix well by vortexing, remove the lids, and immediately load onto the worktable. Click "Next".**

   Note: Avoid freezing and thawing samples more than once or storing samples for over 6 hours at 2–8°C, as this leads to significantly reduced yields of viral nucleic acids.

* Or later version.

9. **The software provides you with the option of entering names for your samples.**
10. **Close the workstation door and start the protocol when instructed by the software. All subsequent steps are automated. The software displays a table of results when the protocol is finished.**
11. **Retrieve the elution tubes containing the purified viral nucleic acids from cooling block 2 at the front of the worktable. The viral nucleic acids are ready to use, or can be stored at 2–8°C for 24 h or at –15 to –30°C to –70°C for longer periods.**

    Note: If heat-labile viral RNA or internal controls have been purified, the post-elution heating step in the MagAttract Virus Mini protocol v1.4 might have a negative impact on RNA integrity. For future sample preparation, use the MagAttract Virus IC protocol v2.2, page 15.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
General handling	
QIAsoft M software error dialog box	If the QIAsoft M software displays an error dialog box during a protocol run, refer to the Troubleshooting Guide in the *BioRobot M48 User Manual*.
Low yield of viral DNA and RNA	
a) MagAttract Suspension B not completely resuspended	Before starting the procedure, ensure that MagAttract Suspension B is fully resuspended. Vortex for at least 3 min before the first use, and for 1 min before subsequent uses.
b) Reagents loaded onto worktable in wrong order	Ensure that all reagents are loaded onto the worktable in the correct order. Repeat the purification procedure with new samples.
c) Carrier RNA not added to Buffer AL	Reconstitute carrier RNA in Buffer AVE and mix with Buffer AL as described on page 10. Repeat the purification procedure with new samples.
d) Degraded carrier RNA	Carrier RNA reconstituted in Buffer AVE was not stored at –15 to –30°C or underwent multiple freeze–thaw cycles. Alternatively, the Buffer AL–carrier RNA mix was stored for over 48 h at 2–8°C. Prepare a new tube of carrier RNA dissolved in Buffer AVE and mix with Buffer AL. Repeat the purification procedure with new samples.
e) Buffer AL–carrier RNA mixed insufficiently	Mix Buffer AL with reconstituted carrier RNA by gently inverting the tube of Buffer AL–carrier RNA mix at least 10 times.

	Comments and suggestions
f) 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.
g) RNA degraded	RNA may have been degraded by RNases in the original plasma or serum samples. Ensure that the samples are processed immediately after collection or removal from storage. Although all buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Take care not to introduce any RNases during the purification procedure or later handling (see Appendix B page 30). Use Buffer AVE for nucleic acid elution.
h) RNase contamination in Buffer AVE	If opening and closing a Buffer AVE vial many times, take care not to introduce RNases. In case of RNase contamination, replace the contaminated vial with a new Buffer AVE vial. Repeat the purification procedure with new samples.
i) Buffer AW1 or AW2 prepared incorrectly	Check that Buffer AW1 or AW2 concentrate was diluted with the correct volume of ethanol, as described on the bottle. Repeat the purification procedure with new samples.
j) Buffer AW1 or AW2 prepared with 70% ethanol	Check that Buffer AW1 or AW2 concentrate was diluted with 96–100% ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the purification procedure with new samples.
k) QIAGEN Protease resuspended in Protease Resuspension Buffer	If samples or internal controls are phosphate-buffered (e.g., PBS, viral transport media, some lyophilized standards), QIAGEN Protease must be resuspended in Buffer AVE instead of Protease Resuspension Buffer. Protease Resuspension Buffer is not compatible with phosphate-buffered starting material.

	Comments and suggestions
DNA or RNA does not perform well in downstream enzymatic reactions	
a) Little or no DNA or RNA in the eluate	See "Low yield of viral DNA and RNA" (above) for possible reasons. Increase the amount of eluate added to the reaction, if possible.
b) Frozen serum or plasma samples not mixed properly after thawing	Thaw frozen samples at room temperature (15–25°C) and mix by pulse vortexing for 15 s.
c) Nucleic acids in samples already degraded prior to purification	Samples were frozen and thawed more than once or stored at room temperature for too long. Always use fresh samples or samples thawed only once (see page 10). Repeat the purification procedure with new samples.
d) Insufficient sample lysis	Reconstituted QIAGEN Protease was stored at elevated temperatures for too long. Repeat the purification procedure using new samples and fresh QIAGEN Protease.
e) Too much or too little carrier RNA in the eluate	Determine the maximum amount of carrier RNA suitable for your amplification reaction. Adjust the concentration of carrier RNA solution added to Buffer AL accordingly (see "Dissolving carrier RNA and adding to Buffer AL", page 10).
f) Too much eluate in the amplification reaction	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly. If the MagAttract Virus IC protocol v2.2 is used, the maximum volume of eluate to be added to the amplification reaction is often significantly higher than after purification with the MagAttract Virus Mini protocol v1.4.
g) Varying performance of purified nucleic acids in downstream assays	The salt and ethanol components of Buffer AW2 may have separated out due to long-term storage. Always mix Buffer AW2 thoroughly before each purification procedure.

	Comments and suggestions
h) New combination of reverse transcriptase and *Taq* DNA polymerase	If the enzymes are changed, it may be necessary to readjust the amount of carrier RNA added to Buffer AL and the amount of eluate used.
i) Carryover of magnetic particles	Carryover of magnetic particles in the eluates will not affect most downstream applications, including RT-PCR. If the risk of magnetic-particle carryover needs to be minimized (e.g., for applications such as real-time PCR), first place the tubes containing eluate in a suitable magnet (e.g., 12-Tube Magnet [cat. no. 36912] for 1 min), and then transfer the eluates to clean tubes. If a suitable magnet is not available, centrifuge the tubes containing eluates in a microcentrifuge at full speed for 1 min to pellet any remaining magnetic particles, and transfer the supernatants to clean tubes.
j) RNA in eluate degraded	If heat-labile viral RNA or internal controls have been purified, the post-elution heating step in the MagAttract Virus Mini protocol v1.4 might degrade the RNA. For future sample preparation, use the MagAttract Virus IC protocol v2.2, page 15.

	Comments and suggestions
k) RNA rich in secondary structures	Some viral RNAs are very rich in secondary structures. A post-elution heating step (e.g., 20 min at 75°C) of open elution tubes leads to significant improvement in performance for certain downstream assays. The post-elution protocol v1.1 on the App. Package, M48, Inf. Dis., v3.2 can be used to perform this heating automatically in the heating/cooling block on the BioRobot M48 worktable. However, for other quantitative downstream assays, this heat incubation has a negative impact. It is recommended to validate the effect of such a post-elution heating step for each individual downstream assay. The MagAttract Virus Mini protocol v1.4 includes a post-elution heating step as an integral part of the protocol while the MagAttract Virus IC protocol v2.2 does not.

Appendix A: Calculating the Amount of Internal Control

To monitor the efficiency of sample preparation and downstream assay, an internal control may need to be added to the sample preparation process. To calculate the amount of internal control (IC) required in MagAttract Virus protocols, the volume of the IC-containing buffer added per sample and the elution volume must be taken into account.

Determining how much internal control will be in downstream reactions

To determine the volume of internal control that will be present in the downstream assay, use the formula:

$$IC_{RXN} = \frac{IC_{AL} \times AL_{SAM} \times EL_{RXN}}{(AL_{TOT} + IC_{AL}) \times EL_{SAM}}$$

where:

IC_{RXN} = Volume of internal control per downstream reaction

IC_{AL} = Volume of internal control added to lysis buffer (AL)

AL_{SAM} = Volume of lysis buffer (AL) per sample

EL_{RXN} = Volume of eluate per downstream reaction

AL_{TOT} = Total volume of lysis buffer (AL) plus carrier RNA used in the protocol

EL_{SAM} = Volume of eluate per sample

As an example, User 1 has added 300 µl of internal control solution (IC_{AL}) to 22.1 ml of lysis buffer (AL_{TOT}) and 152 µl of carrier RNA. Using the MagAttract Virus Mini v1.4 or MagAttract Virus IC v2.2 procedure, 435 µl of lysis buffer will be added per sample (AL_{SAM}), and an elution volume of 75 µl (EL_{SAM}) has been selected by the user. User 1 uses 50 µl of eluate per downstream reaction (EL_{RXN}). The volume of internal control solution in each downstream reaction (IC_{RXN}) is:

$$IC_{RXN} = \frac{300\ \mu l \times 435\ \mu l \times 50\ \mu l}{(22.252\ \mu l + 300\ \mu l) \times 75\ \mu l} = 3.86\ \mu l$$

The final downstream reactions will contain 3.86 µl of internal control solution per reaction.

Determining how much internal control solution to add before starting

Addition of packaged internal control to lysis buffer (AL) for use with the MagAttract Virus Mini protocol v1.4 or MagAttract Virus IC protocol v2.2

If you know the amount of internal control that you want to have present in the downstream assay (IC_{RXN}), then you need to determine the amount to add to the lysis buffer before starting purification (IC_{AL}). To calculate this value, use the formula:

$$IC_{AL} = \frac{IC_{RXN} \times AL_{TOT} \times EL_{SAM}}{(AL_{SAM} \times EL_{RXN}) - (IC_{RXN} \times EL_{SAM})}$$

As an example, User 2 is working with an assay that is optimized for use with 1.0 µl of internal control solution per reaction (IC_{RXN}) and 20 µl of eluate per reaction (EL_{RXN}). User 2 follows the MagAttract protocols with 435 µl of lysis buffer per sample (AL_{SAM}), with a 75 µl elution volume (EL_{SAM}). For 48 samples to be processed, the amount of internal control solution (IC_{AL}) that User 2 needs to add to 22.1 ml lysis buffer plus 152 µl of carrier RNA (AL_{TOT}) is:

$$IC_{AL} = \frac{1.0\ \mu l \times 22252\ \mu l \times 75\ \mu l}{(435\ \mu l \times 20\ \mu l) - (1.0\ \mu l \times 75\ \mu l)} = 194\ \mu l$$

User 2 needs to add 194 µl of internal control solution to 22.1 ml of lysis buffer (AL) plus 152 µl of carrier RNA before purification.

Addition of native internal controls to well 6 of the reaction plate for use with the MagAttract Virus IC protocol v2.2

If you know the amount of internal control that you want to have present in the downstream assay (IC_{RXN}), then you need to determine the amount of internal control to be diluted with Buffer AVE and carrier RNA (IC_{AVE}) before starting the purification. To calculate this value, use the formula:

$$IC_{AVE} = \frac{IC_{RXN} \times IC_{TOT} \times EL_{SAM}}{IC_{SAM} \times EL_{RXN}}$$

where:

IC_{RXN} = Volume of internal control per downstream reaction

IC_{AVE} = Volume of internal control diluted in Buffer AVE–carrier RNA

IC_{SAM} = Volume of diluted internal control added per sample

EL_{RXN} = Volume of eluate per downstream reaction

IC_{TOT} = Total volume of diluted internal control in Buffer AVE–carrier RNA per run

EL_{SAM} = Volume of eluate per sample

As an example, User 3 is working with an assay that is optimized for use with 1.0 µl of internal control solution per reaction (IC_{RXN}) and 20 µl of eluate per reaction (EL_{RXN}). User 3 follows the MagAttract Virus IC protocol v2.2, and a 75 µl elution volume (EL_{SAM}) has been selected. For each processed sample, a volume of 60 µl of diluted internal control has to be manually pipetted into well 6 of the reaction plate but during the sample preparation process of the MagAttract Virus IC protocol the BioRobot will only transfer 30 µl of diluted internal control (IC_{SAM}) from well 6 to the binding reaction. For 48 samples being processed in one run, the total volume of diluted internal control (IC_{TOT}) to be made is:

IC_{TOT} = Number of samples per run x 60 µl

= 48 x 60 µl = 2880 µl

The volume of internal control solution (IC_{AVE}) that User 3 needs for 48 samples is:

$$IC_{AVE} = \frac{1.0\ \mu l \times 2880\ \mu l \times 75\ \mu l}{(30\ \mu l \times 20\ \mu l)} = 360\ \mu l$$

To obtain a final concentration of 10 ng/µl carrier RNA, the volume of carrier RNA stock solution with 1 µg/µl to be added to the IC dilution has to be calculated:

Volume of carrier RNA stock = IC_{TOT} x 10 ng/µl / 1000ng/µl
= 2880 µl / 100 = 28.8 µl

For a final total volume of 2880 µl of diluted internal control, the user has to add Buffer AVE:

Volume of Buffer AVE = IC_{TOT} – IC_{AVE} – Volume of carrier RNA
= 2880 µl – 360 µl – 28.8 µl = 2491 µl

User 3 needs to add 360 µl of internal control solution to 2491 µl Buffer AVE and 28.8 µl of carrier RNA stock to obtain 2880 µl of diluted internal control. From this diluted internal control, 60 µl has to be manually transferred into well 6 of each reaction plate before the MagAttract Virus IC Protocol v2.2 is started.

Appendix B: General Remarks on Handling RNA

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 even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure. To create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non-disposable vessels and solutions while working with RNA.

General handling

Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible. Keep purified RNA on ice when aliquots are pipetted for downstream applications.

Disposable plasticware

The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases.

Non-disposable plasticware

Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH,* 1 mM EDTA* followed by RNase-free water (see "Solutions", page 31). 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 a detergent,* thoroughly rinsed, and oven baked at 240°C for 4 hours or more (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight (12 hours) at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

Electrophoresis tanks

Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS),* thoroughly rinsed with RNase-free water, and then rinsed with ethanol† and allowed to dry.

Solutions

Solutions (water and other solutions)* should be treated with 0.1% DEPC. 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. Add 0.1 ml DEPC to 100 ml of the solution to be treated and shake vigorously to bring the DEPC into solution. Let the solution incubate for 12 hours at 37°C. Autoclave for 15 minutes to remove any trace of 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. 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 RNA:RNA or RNA:DNA hybrids is not seriously affected unless a large fraction of the purine residues have been modified. Residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for 15 minutes.

Note: MagAttract Virus Mini M48 Kit buffers are guaranteed RNase-free without using DEPC treatment and are therefore free of any DEPC contamination.

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

† Plastics used for some electrophoresis tanks are not resistant to ethanol. Take proper care and check the supplier's instructions.

Ordering Information

Product	Contents	Cat. no.
MagAttract Virus Mini M48 Kit (192)	For 192 viral nucleic acid preps: MagAttract Suspension B, RNA-Free Reagents and Buffers	955336
App. Package, M48, Inf. Dis.	Software protocol package for infectious disease applications, v. 3.2, on the BioRobot M48 workstation	9016145
Accessories		
QIAGEN Protease (7.5 AU)	7.5 Anson units per vial (lyophilized)	19155
QIAGEN Protease (30 AU)	4 x 7.5 Anson units (lyophilized)	19157
Buffer AL (216 ml)	216 ml of Buffer AL	19075
Buffer AW1 (concentrate, 242 ml)	242 ml of Buffer AW1 concentrate	19081
Buffer AW2 (concentrate, 324 ml)	324 ml of Buffer AW2 concentrate	19072
Buffer AVE (108 x 2 ml)	108 x 2 ml of Buffer AVE	1020953
Carrier RNA (12 x 1350 µg)	12 x 1350 µg of Carrier RNA	1017647
Starter Pack, M48	Pack includes: 600 sterile filter-tips; 40 sample prep plates; 8 large reagent containers; 8 small reagent containers; 8 silicon seals; 250 sample tubes, 1.5 ml; 250 sample tubes, 2 ml; 250 elution tubes, screw cap, 1.5 ml; 2 tip waste bags	995999
Filter-Tips, 1000 µl, M48 (1000)	Sterile, disposable filter-tips, bagged; pack of 1000	995652
Reagent Containers, small, M48 (100)	Reagent containers (20 ml) with lids. To be used with the Reagent Container Rack, M48; pack of 100	995902
Reagent Containers, large, M48 (50)	Reagent containers (110 ml) with lids. To be used with the Reagent Container Rack, M48; pack of 50	995904

Product	Contents	Cat. no.
Reagent Container Seals, M48 (50)	Lid-sealing sheets for small and large reagent containers, allowing storage of unused reagents; pack of 50	995906
Sample Prep Plates, 42-well, M48 (100)	Disposable polypropylene plates for sample preparation, including nucleic acid binding and washing steps; pack of 100	995908
12-Tube Magnet	Magnet for separating magnetic particles in 12 x 1.5 ml or 2 ml tubes	36912
Related products		
MagAttract Viral RNA M48 Kit — for automated viral RNA purification from up to 300 µl serum or plasma samples using the BioRobot M48 workstation		
MagAttract Viral RNA M48 Kit (96)	For 96 viral RNA preps: MagAttract Suspension F, RNase-Free Buffers	955235
EZ1 Virus Mini Kit — for automated, simultaneous purification of viral DNA and RNA from 1–6 serum and plasma samples using the BioRobot EZ1 workstation		
EZ1 Virus Mini Kit v2.0 (48)	For 48 preps: Reagent Cartridges (Virus Mini v2.0), Disposable Filter-Tips, Disposable Tip-Holders, Sample Tubes (2 ml), Elution Tubes (1.5 ml), Buffer AVE, Carrier RNA	955134
EZ1 Virus Card v2.0	Preprogrammed card for EZ1 Virus v2.0 purification protocols	9017330
EZ1 Advanced Virus Card v2.0	Preprogrammed card for purification of viral DNA and RNA using the EZ1 Advanced	9018303
EZ1 Advanced XL Virus Card v2.0	Preprogrammed card for purification of viral DNA and RNA using the EZ1 Advanced XL	9018708

Product	Contents	Cat. no.
QIAamp® UltraSens® Virus Kit — for concentration and purification of viral RNA and DNA from serum and plasma		
QIAamp UltraSens Virus Kit (50)*	For 50 viral nucleic acid preps: 50 QIAamp Mini Spin Columns, Proteinase K, Carrier RNA, Collection Tubes (2 ml), Buffers	53704
QIAamp MinElute® Virus Kits — for simultaneous purification of viral RNA and DNA from plasma, serum, and cell-free body fluids		
QIAamp MinElute Virus Spin Kit (50)	For 50 minipreps: 50 QIAamp MinElute Columns, QIAGEN Protease, Carrier RNA, Buffers, Collection Tubes (2 ml)	57704
QIAamp MinElute Virus Vacuum Kit (50)†	For 50 minipreps: 50 QIAamp MinElute Columns, QIAGEN Protease, Carrier RNA, Buffers, Extension Tubes (3 ml), Collection Tubes (1.5 ml)	57714
QIAamp Viral RNA Mini Kit — for purification of viral RNA from cell-free body fluids		
QIAamp Viral RNA Mini Kit (50)*‡	For 50 RNA preps: 50 QIAamp Mini Spin Columns, Carrier RNA, Collection Tubes (2 ml), RNase-free Buffers	52904
QIAamp Virus BioRobot MDx Kit — for automated purification of viral RNA and DNA from cell-free body fluids using the BioRobot MDx workstation		
QIAamp Virus BioRobot MDx Kit (12)	For 12 x 96 preps: 12 QIAamp 96 Plates, RNase-free Buffers, QIAGEN Protease, Elution Microtubes CL, Caps, S-Blocks, Carrier RNA	965652

* Other kit sizes are available; see www.qiagen.com.

† Requires use of a vacuum manifold, such as QIAvac 24 Plus with VacConnectors (see page 36).

‡ QIAamp Mini spin columns can be used either in a microcentrifuge or on vacuum manifolds (QIAvac 24 with VacConnectors and VacValves, see page 36).

Product	Contents	Cat. no.
QIAvac vacuum manifolds and accessories — for vacuum-driven processing of spin columns		
QIAvac 24 Plus	Vacuum manifold for processing 1–24 spin columns: includes QIAvac 24 Plus Vacuum Manifold, Luer Plugs, Quick Couplings	19413
VacConnectors (500)	500 disposable connectors for use with QIAamp spin columns on luer connectors	19407
VacValves (24)	24 valves for use with the QIAvac 24 and QIAvac 24 Plus	19408
Vacuum Pump	Universal vacuum pump (capacity 34 L/min, 8 mbar vacuum abs.)	84000* 84010† 84020‡
QIAvac Connecting System	System to connect vacuum manifold with vacuum pump: includes Tray, Waste Bottles, Tubings, Couplings, Valve, Gauge, 24 VacValves	19419
Vacuum Regulator	For use with QIAvac manifolds	19530

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.

* Japan.

† US and Canada.

‡ Rest of World.

Notes

Notes

Limited License Agreement for MagAttract Virus Mini M48 Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

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1095814 06/2015

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