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MagAttract® Viral RNA Kit Handbook

For hands-free isolation of RNA from respiratory swab samples using automated processing and liquid-handling systems

Sample to Insight

QIAGEN

Contents

Kit Contents

MagAttract Viral RNA Kit **Catalog no.** **Number of preps**	**(960)** **955538** **960**
Proteinase K	3 x 2 ml
Buffer ACL	1 x 220 ml
Carrier RNA (1350 µg)	16
QSB1 concentrate	2 x 95 ml
MagAttract Suspension G	2 x 13 ml
MW1 concentrate	3 x 180 ml
Buffer AVE	1 x 125 ml
Quick-Start Protocol	1

Shipping and Storage

The MagAttract Viral RNA Kit is shipped at room temperature. All kit components of the MagAttract Viral RNA Kit can be stored at room temperature (15–25°C). Under these conditions, the components are stable for 12 months without showing any reduction in performance and quality, unless otherwise indicated on the label.

Intended Use

All MagAttract products are intended for molecular biology applications. These products are 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.

CAUTION 	QSB1 and MW1 are flammable.

CAUTION 	DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Solution ACL, QSB1, and MW1 contain chaotropic salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a 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.

Quality Control

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

Introduction

The MagAttract Viral RNA Kit allows automated high-throughput isolation of viral RNA from up to 960 human respiratory swab samples in less than 1 day and is optimized for use with the Thermo Scientific® KingFisher® Flex platform. Protocols for other instruments are being developed; please contact QIAGEN Technical Services if you are interested in using a different instrument with this kit.

Warnings and precautions

RNA is extremely sensitive to ribonucleases (RNases) and should always be prepared with due care. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Please read "Appendix: General Remarks on Handling RNA" (page 15) before starting. PCR should always be carried out using good laboratory practices. Accordingly, a PCR laboratory should always be divided into 3 areas: reagent preparation, sample preparation, and amplification and detection. The high sensitivity of PCR necessitates that all reagents remain pure and uncontaminated; they should be monitored carefully and routinely. Contaminated reagents must be discarded.

Handling of 96-well plates

The sensitivity of nucleic acid amplification technologies necessitates the following precautions when handling 96-well plates to avoid cross-contamination between sample preps:

- Carefully apply the sample or solution to the 96-well plate. Pipet the sample into the center of the respective well.
- Change pipette tips between all liquid transfer steps. The use of aerosol-barrier tips is recommended.
- Wear gloves throughout the procedure. In case of contact between gloves and sample, change gloves immediately.

Principle and procedure

The MagAttract Viral RNA Kit combines the speed and efficiency of silica-based nucleic acid purification with the convenient handling of magnetic particles. A well-established technology is used for viral RNA preparation in a 96-well format. The purification procedure is designed to ensure reproducible handling and comprises 4 steps: lyse, bind, wash, and elute.

High-quality RNA is eluted in a special RNase-free buffer, ready for direct use or safe storage. The purified RNA is free of proteins, nucleases, and other contaminants or inhibitors and can be used immediately in demanding downstream applications, including PCR, qPCR, dPCR, and next-generation sequencing.

Starting material

The standard starting materials for the MagAttract Viral RNA Kit are respiratory swab samples. QIAGEN has tested compatibility to this sample material. Other sample materials may be compatible with the kit. Please refer to the product page for up-to-date information on sample materials that have been tested by QIAGEN.

Handling of starting material

Swabs should be placed immediately into a sterile transport tube containing either UTM (universal transport medium), VTM (viral transport medium), liquid amies, or sterile 0.9% saline.

Carrier RNA

Carrier RNA serves two purposes: first, it enhances binding of viral nucleic acids to the magnetic beads, 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. Not adding carrier RNA to Buffer ACL may lead to reduced viral RNA recovery.

The amount of lyophilized carrier RNA provided is sufficient for the volume of Buffer ACL supplied with the kit. Carrier RNA concentration has been adjusted so that the MagAttract Viral RNA Kit can be used as a generic purification system compatible with many different amplification systems.

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. Therefore, calculations of how much eluate to add to downstream amplifications should consider 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 ACL.

Elution with Buffer AVE

Buffer AVE is RNase-free water that contains 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. If determination of RNA purity in eluate by spectrophotometric analysis is required, we recommend elution with RNase-free water instead of Buffer AVE.

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.

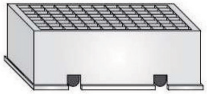
- Multichannel pipettor (50–1000 µl)
- 80% ethanol
- Multichannel pipettor reagent reservoirs for 10–150 ml
- KingFisher Flex consumables (needed at least for 960 samples if all wells of 96-well plates are used): 10 combs, 40 x 96 deep-well plates, and 10 x 96 microplates:

ThermoFisher	
Description	**Cat. no.**
KingFisher 96 deep-well plate, v-bottom, polypropylene	95040450
KingFisher 96 tip comb for deep-well magnets, 10 x 10 pcs/box	97002434
KingFisher 96 microplate (200 µl), 48 pieces	97002540

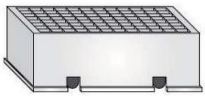
Important Notes

If preparing RNA for the first time, see "Appendix: General Remarks on Handling RNA", page 15). All steps of the MagAttract Viral RNA protocol should be performed quickly and at room temperature (15–25°C).

Offboard lysis:



Proteinase K added to KingFisher 96 deep-well plate
Sample added



MagAttract suspension prepared and added

Bind, wash, end Elute steps on KingFisher Flex work deck

Protocol: MagAttract Viral RNA Kit on KingFisher Flex

This protocol describes the use of the MagAttract Viral RNA Kit with the KingFisher Flex instrument.

Things to do before starting

- Download the software protocol from MagAttract Viral RNA product page and install it on KingFisher Flex instrument.
- Prepare Buffer QSB1 and Buffer MW1 according to the instructions on the bottles.
- Ethanol (80%) is required in this protocol and needs to be supplied by the user.
- Add carrier RNA, reconstituted in Buffer AVE, to Buffer ACL (2 ml carrier RNA–AVE solution to 15 ml Buffer ACL per each 96-well plate that will be processed). Dissolve the carrier RNA thoroughly and if necessary, divide it into conveniently sized aliquots, and store it at –30 to –15°C.
  Note: Do not freeze–thaw the aliquots of carrier RNA more than 3 times.

Procedure

1. Start the KingFisher Flex instrument and select the MagAttract Viral RNA protocol (see "Things to do before starting").

2. Add 5 µl Proteinase K per well of a clean KingFisher 96 deep-well plate (user provided).

3. Transfer 300 µl sample to the 96-well plate.

4. Resuspend MagAttract Suspension G Beads by vortexing. For each complete 96-well plate to be processed, prepare a mixture of 17 ml Buffer ACL containing carrier RNA (see "Things to do before starting"), 27.5 ml Buffer QSB1, and 2.5 ml of MagAttract Suspension G. Immediately transfer to a multichannel pipette reservoir.

   Note: Maintain the MagAttract Suspension G Beads in suspension to ensure uniform distribution.

5. Add 470 µl of the mix from step 4 with a multichannel pipette to each well of the KingFisher 96-well plate from step 3.

6. Place the sample plate on the work deck of the KingFisher Flex instrument at the specified location as indicated on the instrument display.

7. Prepare four KingFisher 96-well plates (user provided):

   7a. Add 500 µl of Buffer MW1 into each well of one KingFisher 96 deep-well plate.

   7b. Add 500 µl of 80% ethanol (user provided) into each well of two KingFisher 96 deep-well plates.

   7c. Add 80 µl Buffer AVE into each well of one KingFisher 96 microplate.

   Place each plate on the deck as indicated on the instrument display.

8. Initiate the protocol run.

9. Upon completion, cover the wells of the KingFisher 96 microplate containing eluate with an appropriate storage seal (user provided). The RNA is now ready for downstream applications.

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 in 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 or protocols in this handbook (for contact information, visit **support.qiagen.com**).

		Comments and suggestions
Little or no RNA in the eluate		
a)	Sample frozen and thawed more than once	Avoid repeated freezing and thawing. Always use fresh samples or samples thawed only once.
b)	Frozen samples were not mixed properly after thawing	Thaw frozen samples with mild agitation to ensure thorough mixing.
c)	Magnetic particles were not completely resuspended	Before starting the procedure, ensure that the magnetic particles are fully resuspended. Vortex for at least 3 min before use.
d)	Carrier RNA not added	Add carrier RNA into Buffer ACL and repeat the purification procedure with new samples.
e)	RNA degraded	Often RNA is degraded by RNases in the starting material. Ensure that the samples are processed quickly. If necessary, add RNase inhibitor to the sample. Check for RNase contamination of buffers and water, and ensure that no RNase is introduced during the procedure.
f)	Buffer QSB1 or MW1 prepared incorrectly	Check that Buffer QSB1 and Buffer MW1 concentrates were diluted with correct volumes of pure (96–100%) ethanol. Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone. Repeat the purification procedure with a new sample.

		Comments and suggestions
RNA does not perform well in subsequent enzymatic reactions		
a)	Little or no RNA in the eluate	Check "Little or no RNA in the eluate" (above) for possible reasons.
b)	Too much carrier RNA in the eluate	Determine the maximum amount of carrier RNA suitable for your RT-PCR. Adjust accordingly the concentration of carrier RNA added to Buffer ACL.
c)	Reduced sensitivity	Determine the maximum volume of eluate suitable for your RT-PCR. Reduce the volume of eluate added to the RT-PCR
d)	New combination of reverse transcriptase and Taq DNA polymerase used	If enzymes are changed, it may be necessary to readjust the amount of carrier RNA solution added to Buffer ACL.
e)	Cross-contamination between samples	To avoid cross-contamination when handling 96-well plates, follow the guidelines in "Handling of 96-well plates" on page 6. Repeat the purification procedure with new samples.

Appendix: General Remarks on Handling RNA

RNases are very stable and active enzymes. RNases are very stable and therefore difficult to inactivate. Even minimal amounts are sufficient to destroy RNA. Make sure that every plasticware or glassware that comes into contact with the solution has undergone sufficient RNase decontamination. 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, take the following precautions during pretreatment and use of disposable and nondisposable 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. RNase contamination from bench surfaces, nondisposable plasticware, and laboratory equipment (e.g., pipettes and electrophoresis tanks) can be removed using general laboratory reagents. To decontaminate plasticware, rinse with 0.1 M NaOH, 1 mM EDTA* followed by RNase-free water or rinse with chloroform* if the plasticware is chloroform resistant. To decontaminate electrophoresis tanks, clean with detergent (e.g., 0.5% SDS),* rinse with RNase-free water, rinse with ethanol (if the tanks are ethanol resistant), and allow to dry.* 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.

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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.

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 at least 4 hours (overnight, if more convenient) before use. Autoclaving alone will not fully inactivate many RNases. Alternatively, glassware can be treated with DEPC* (diethyl pyrocarbonate).

Solutions

Solutions should be purchased RNase-free or treated with 0.1% DEPC. We recommend purchasing RNase-free water, because if trace amounts of DEPC remain after autoclaving buffer, purine residues in RNA might be modified by carbethoxylation, and performance of enzymatic reactions such as PCR may be negatively affected. Therefore, residual DEPC must always be eliminated from solutions or vessels by autoclaving or heating to 100°C for at least 15 minutes.

* 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.

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. 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, use RNase-free water to 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.

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Ordering Information

Product	Contents	Cat. no.
MagAttract Viral RNA Kit (960)	For hands-free isolation of RNA from respiratory swab samples using automated processing and liquid-handling systems	955538
Related products		
Buffer ACL (220 ml)	220 ml lysis buffer for off-board lysis protocols	939017
MagAttract Suspension G (13 ml)	13 ml MagAttract Suspension G	1026901
QIAGEN Proteinase K (2 ml)	For protease digestion during DNA and RNA preparation	19131
Carrier RNA	Carrier RNA (poly A) 12 x 1350 µg	1017647
MagAttract Blood DNA/RNA Kit (384)	For the automated isolation of blood DNA using a KingFisher Flex or Duo	22100-4-KF
MagAttract Power Soil DNA KF Kit	For the automated, hands-free isolation of DNA from soil	27000-4-KF

MagAttract Power Microbiome DNA/RNA KF	For the automated, hands-free isolation of nucleic acids from stool and gut material	27600-4-KF
MagAttract PowerClean DNA Kit	For the automated removal of PCR inhibitors from previously purified DNA using magnetic bead technology	27900-4-KF

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

Date	Changes
06/2021	Initial release.

Notes

Notes

Limited License Agreement for MagAttract Viral RNA Kit

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

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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