applied biosystems

MagMAX™-96 AI/ND Viral RNA Isolation Kit

USER GUIDE

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For Laboratory Use Including Veterinary and Environmental Uses.

ThermoFisher SCIENTIFIC

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Revision	Date	Description
E	22 March 2018	Update licensing, trademarks, general style and format.
D	7 January 2008	Baseline for this revision history.

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1 Product information

Product description

The MagMAX™-96 AI/ND Viral RNA Isolation Kit is designed for rapid high throughput purification of Avian Influenza (AI) and/or Newcastle Disease (ND) viral RNA from pharynx/tracheal and cloacal swab samples in 96-well plates. This kit is validated by the National Veterinary Services Laboratories for use in their testing protocol for real-time RT-PCR detection of Avian Influenza virus and Newcastle Disease virus in clinical samples. For isolation of a broader range of nucleic acids from biofluid samples, we recommend the MagMAX™ Viral RNA Isolation Kit (Cat. No. AM1939) and the MagMAX™-96 Viral RNA Isolation Kit (Cat. No. AM1836).

The microspherical paramagnetic beads that are used in the kit have a large available binding surface and can be fully dispersed in solution, allowing thorough nucleic acid binding, washing, and elution. The procedure, therefore, delivers consistent yields of high-quality RNA with little sample-to-sample variation.

96 samples can be processed at one time with the MagMAX™-96 AI/ND Viral RNA Isolation Kit. However, it can also be used to isolate RNA from fewer than 96 samples. For viral nucleic acid isolation from whole blood, or cell culture and tissue samples, we recommend the MagMAX™-96 Blood RNA Isolation Kit (Cat. No. AM1837) andMagMAX™-96 Total RNA Isolation Kit (Cat. No. AM1830), respectively.

Contents and storage

Table 1 MagMAX™-96 AI/ND Viral RNA Isolation Kit (Cat. No. AM1835)

Contents	Amount	Storage
Nuclease-free Water	50 mL	15–30°C
Processing Plates and Lids	4 plates	15–30°C
Viral Lysis/Binding Soln Concentrate[1]	50 mL	15–30°C[2]
Bead Resuspension Solution[1]	10 mL	15–30°C[2]
Wash Solution 1 Concentrate[1]	105 mL	15–30°C
Wash Solution 2 Concentrate[1]	100 mL	4–30°C
Elution Buffer	20 mL	4–30°C

Contents	Amount	Storage
RNA Binding Beads[1]	2 mL	4°C[2]
Carrier RNA	500 µL	–20°C

[1] See "Before first use of the kit" on page 10 for preparation instructions.
[2] Do not freeze.

Required materials not supplied

Item	Source
(*Optional*) Automation instruments, one of the following[1]:	
KingFisher™ 96 Magnetic Particle Processor	Contact your local sales office.
KingFisher™ Flex Magnetic Particle Processor	
MagMAX™ Express-96 Magnetic Particle Processor	
Equipment	
Orbital shaker (96-well plates)	MLS
Magnetic stand, one of the following: • Magnetic-Ring Stand (96 well) (*recommended*) • Magnetic Stand-96	• AM10050 • AM10027
Plates, tips and accessories	
96-well U-bottom plates and lids	**thermofisher.com/plastics**
RNAse-free pipette tips	**thermofisher.com/pipettetips**
Reagents	
100% ethanol, ACS grade or better	MLS
100% isopropanol, ACS grade or better	MLS
RNase*Zap*™ RNase Decontamination Solution	AM9780

[1] If using a different instrument, the robotic liquid handler must have a 200 µL pipetting tool, gripper tool, six reservoirs with reservoir holders, a magnetic stand for 96-well plates, and an integrated orbital shaker.

Overview of the procedure

Classic viral particle disruption and magnetic bead-based RNA purification

The MagMAX™-96 AI/ND Viral RNA Isolation Kit uses a classic method for disrupting viral particles in a guanidinium thiocyanate-based solution that rapidly releases viral RNA while simultaneously inactivating nucleases in the sample matrix (Figure 1 on page 7). Paramagnetic beads with a nucleic acid binding surface are then added to the sample to bind nucleic acids. The beads/nucleic acids are captured on magnets, and proteins and other contaminants are washed away. The beads are then washed again to remove remaining binding solutions. RNA is eluted in a small volume of Elution Buffer.

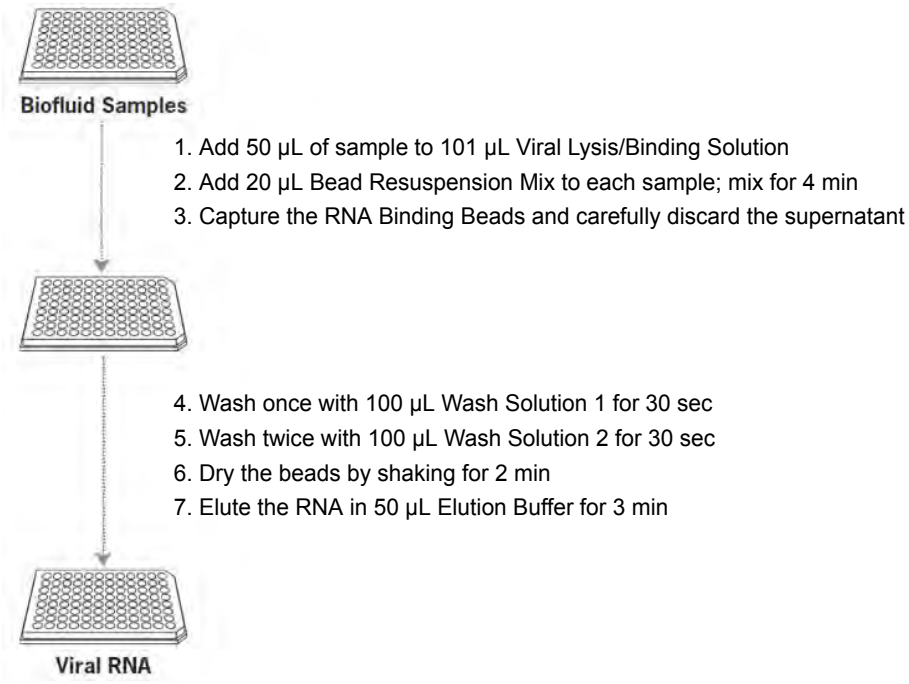

Figure 1 MagMAX™-96 AI/ND viral RNA isolation procedure

Sample size and RNA recovery

The MagMAX™-96 AI/ND Viral RNA Isolation Kit can efficiently isolate viral RNA from biofluid samples as large as 50 µL containing as few as 20 RNA copies. RNA recovery is dependant on sample type and is typically greater than 75%. The RNA recovered with the kit is of high quality and purity, and is appropriate for real-time RT-PCR. Figure 2 shows an example of qRT-PCR data that illustrates linear RNA recovery using the kit from several different samples with a broad range of RNA inputs.

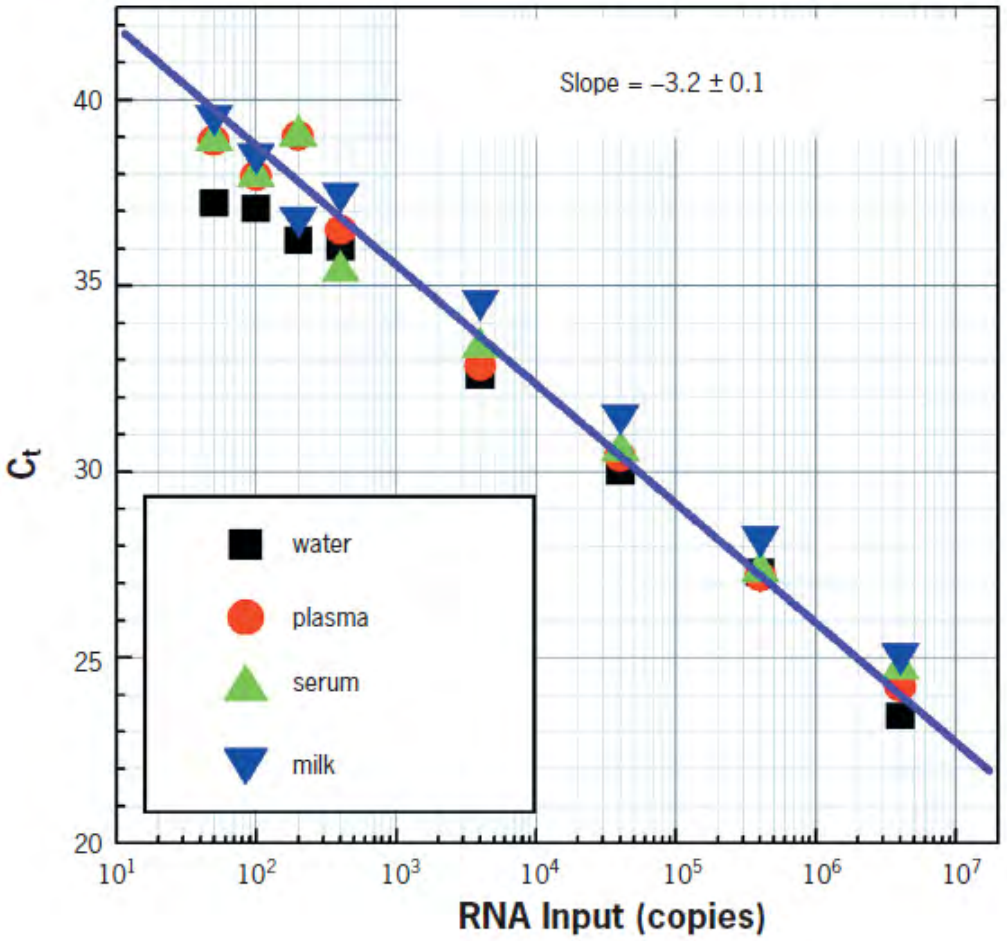

Figure 2 Recovery of viral RNA using MagMAX™-96 AI/ND Viral RNA Isolation Kit
Serial dilutions of HIV Armored RNA® transcripts were spiked into water, plasma, serum, and milk. Viral RNA was isolated using the MagMAX™-96 AI/ND Viral RNA Isolation Kit according to the protocol. Equivalent volumes of recovered viral RNA (1/5 of eluted volume) were used in a 15 µL qRT-PCR to detect the HIV transcript.

Manual or robotic high throughput processing

MagMAX™-96 AI/ND Viral RNA Isolation Kit is optimized both for robotic liquid handlers, and for use manually with 96-well plates and multichannel pipettors. Visit the resource page for detailed automation instructions and downloadable protocols for select robotic platforms: **www.thermofisher.com/us/en/home/life-science/dna-rna-purification-analysis/automated-purification-extraction/automated-protocols-software.html**.

2 Methods

Guidelines for RNA isolation

- Pharynx/tracheal and cloacal swabs that are collected in BHI or VTM media are the recommended sample type. Use up to 50 µL sample per prep.
- 96 samples can be processed at one time. To process fewer samples at a time, additional polystyrene U-bottom 96-well plates and lids are needed.
- Before working with RNA, clean the lab bench and pipettors with an RNase decontamination solution (for example, RNase*Zap*™ RNase Decontamination Solution).
- Wear laboratory gloves for this procedure. Gloves protect you from the reagents, and they protect the RNA from nucleases that are present on skin.
- Use RNase-free pipette tips to handle the kit reagents, and avoid putting used tips into the reagent containers.

Before you begin

Determine the maximum plate shaker setting

Using 180 µL of water per well, determine the maximum shaking speed that can be used with your orbital shaker without spilling sample. Use this speed for all shaking steps.

Before first use of the kit

1. Add Carrier RNA to Viral Lysis/Binding Solution Concentrate according to the table below, and mix briefly. Then add 100% isopropanol and mix well. Note that 10% overage is included in solution preparation instructions for more than one reaction. If you prepare the entire bottle, mark the label to indicate that the Carrier RNA and isopropanol were added.

Component	Volume per reaction	Volume per plate	Volume per bottle
a. Combine the following:			
Viral Lysis/Binding Solution Concentrate	50 µL	6.25 mL	25 mL
Carrier RNA	1 µl	125 µL	500 µL
b. Mix briefly, then add:			
100% Isopropanol	50 µL	6.25 mL	25 mL
c. Mix well by vortexing.			

IMPORTANT! Shipment on dry ice may cause the Carrier RNA to become gelatinous and difficult to pipet. If you experience problems when attempting to pipet the Carrier RNA, we recommend heating it in a hybridization oven at 37°C for 10–15 min. A heat block may be used for this incubation, as long as the block accommodates the tube to uniformly heat the solution to 37°C. After heating, vortex vigorously, then spin briefly; you should now be able to easily pipette the solution accurately.

IMPORTANT! Prepared Viral Lysis/Binding Solution is stable at room temperature for one month. Prepare only the amount of Viral Lysis/Binding Solution needed for one month by scaling the reagent volumes down proportionally if necessary. Do not store prepared Viral Lysis/Binding Solution at 4°C or below as this may cause the Carrier RNA to precipitate; if the solution is inadvertently stored at 4°C, warm it at 37°C and shake to dissolve any precipitates before use.

2. Add 35 mL 100% isopropanol to the bottle labeled Wash Solution 1 Concentrate and mix well. Mark the label to indicate that the isopropanol was added. The resulting mixture is called Wash Solution 1.

3. Add 80 mL 100% ethanol to the bottle labeled Wash Solution 2 Concentrate and mix well. Mark the label to indicate that the ethanol was added. The resulting mixture is called Wash Solution 2.

4. Dilute the Bead Resuspension Solution with Nuclease-free Water according to the table below, and mix briefly. Then add the RNA Binding Beads, and mix again. Finally, add the 100% isopropanol and mix thoroughly by vortexing. Note that 10% overage is included in solution preparation instructions for more than one reaction. This mixture is called Bead Resuspension Mix.

Component	Volume per reaction	Volume per plate	Volume per bottle
a. Combine the following:			
Bead Resuspension Solution	6 µl	750 µL	3 mL
Nuclease-free water	4 µL	500 µL	2 mL
b. Mix briefly, then add:			
RNA Binding Beads[1]	4 µL	500 µL	2 mL
c. Mix briefly, then add:			
100% Isopropanol	6 µl	750 µL	3 mL
d. Mix well by vortexing.			

[1] Mix the RNA Binding Beads thoroughly by vortexing before dispensing.

IMPORTANT! Bead Resuspension Mix can be stored at room temperature for up to one month. If necessary, scale the reagent volumes down proportionally to prepare the amount of Bead Resuspension Mix needed for one month.

Store all of the prepared reagents at room temperature.

Isolate the RNA

The protocol is for the manual isolation of RNA. See "Isolate the RNA: automated protocol overview" on page 16 for the automated protocol using the KingFisher™ Purification System (for 1–24 samples per run) or the KingFisher™ 96 Magnetic Particle Processor.

Use the maximum shaker speed that is identified in "Determine the maximum plate shaker setting" on page 10 for all the shaking steps in the protocol.

1. Add 101 µL prepared Viral Lysis/Binding Solution (Carrier RNA and isopropanol added) to each well of the Processing Plate.

2. Transfer up to 50 µL of sample to each well of the Processing Plate containing Viral Lysis/Binding Solution.

   When adding sample, immerse pipette tips slightly in the Viral Lysis/Binding Solution to prevent creating aerosols that can lead to cross-contamination.

3. Shake the Processing Plate for 30 seconds on an orbital shaker.

4. Vortex the Bead Resuspension Mix to resuspend the beads before pipetting (prepared in "Before first use of the kit" on page 10).

5. Add 20 µL Bead Resuspension Mix to each sample in the Processing Plate.

6. Shake the Processing Plate for 4 minutes on an orbital shaker to lyse viruses and bind RNA to the RNA Binding Beads.

7. Move the Processing Plate to a magnetic stand to capture the RNA Binding Beads for 2 minutes, or until the mixture becomes clear. When capture is complete, the RNA Binding Beads form pellets against the magnets in the magnetic stand. The capture time depends on the magnetic stand used.

8. Carefully aspirate, then discard the supernatant without disturbing the beads.

   IMPORTANT! To obtain pure RNA, it is important to remove completely the supernatant at this step. Use the Magnetic-Ring Stand (96 well) for the best consistency.

9. Remove the Processing Plate from the magnetic stand.

10. Add 100 µL Wash Solution 1 (isopropanol added) to each sample, then shake the plate for 30 seconds.

    The RNA Binding Beads may not fully disperse during this step. It will not affect RNA purity or yield.

11. Move the Processing Plate to a magnetic stand to capture the RNA Binding Beads for 1 minute, or until the mixture becomes clear.

12. Carefully aspirate, then discard the supernatant without disturbing the beads.

13. Remove the Processing Plate from the magnetic stand.

14. Perform 2 washes with 100 µL Wash Solution 2 (ethanol added).
    a. Add 100 µL Wash Solution 2 to each sample, then shake the Processing Plate for 30 seconds.

    b. Move the Processing Plate to a magnetic stand to capture the RNA Binding Beads for 1 minute, or until the mixture becomes clear.

    c. Carefully aspirate, then discard the supernatant without disturbing the beads.

    d. Repeat with a second 100 µL of Wash Solution 2.

       IMPORTANT! To obtain pure RNA, it is important to remove completely the supernatant at this step.

15. If there is remaining solution in any of the wells in sufficient volume to remove with a fine-tipped pipette, do so before moving the plate onto the shaker.

16. Move the Processing Plate to the shaker, then shake vigorously for 2 minutes to allow any remaining alcohol from the Wash Solution 2 to evaporate.

    IMPORTANT! Do not shake the plate for >5 minutes, because this could over dry the beads.

17. Add 50 µL Elution Buffer (room temp or prewarmed to 37–65°C) to each sample, then shake for 3 minutes.

    Note: The RNA can be eluted in as little as 20 µL. The volume of Elution Buffer that is supplied with the kit is sufficient for 4 × 96 samples at 50 µL each.

18. Move the Processing Plate to a magnetic stand to capture the RNA Binding Beads for 1 minute, or until the mixture becomes clear.
    The purified RNA is in the supernatant.

19. Transfer the supernatant, which contains the RNA, to a nuclease-free container appropriate for your application.

    Note: Leave 1–2 µL liquid behind to minimize bead carryover into the storage plate.

Store the purified RNA at –20°C.

Guidelines for viral RNA analysis

Detect viral RNA by real-time RT-PCR

This kit is designed for purification of AI/ND RNA for RT-PCR amplification. Quantitative real-time RT-PCR is a powerful method for viral RNA detection and is the recommended analysis tool. Visit **thermofisher.com/cdna** and **thermofisher.com/qpcr** for more information.

Quantify recovered Carrier RNA

The viral RNA recovered from most samples is present in limited amounts. Most RNA in the purified sample is the Carrier RNA that was added to the Viral Lysis/Binding Solution. RNA recovery is heavily dependent on sample type (for example, plasma vs. swab samples). With most sample types, up to 75% of Carrier RNA is be recovered. Using the recommended volume (101 µL) of prepared Lysis/Binding Solution, each sample contains approximately 1 µg Carrier RNA. Therefore >5 ng/µL RNA is be recovered.

Quantify the amount of Carrier RNA by UV absorbance at 260 nm (A_{260}) using the NanoDrop™ 2000/2000c Spectrophotometer. Absorbance readings using the NanoDrop™ are fast and easy because 1.5 µL can be measured without dilution, and no cuvettes are needed.

Alternatively, the RNA concentration can be determined by diluting an aliquot of the preparation in TE (10-mM Tris-HCl pH 8, 1 mM EDTA), and reading the absorbance in a traditional spectrophotometer at 260 nm. Find the concentration in µg/mL by multiplying the A_{260} by the dilution factor and the extinction coefficient. (1 A_{260} = 40 µg RNA/mL).

A_{260} × dilution factor × 40 = µg RNA/mL

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Troubleshooting

Poor viral nucleic acid detection

If poor or no viral RNA signal is observed by RT-PCR (i.e., the C_t is higher than expected), it could be due to inhibitors in the nucleic acid recovered or poor nucleic acid recovery.

Inhibitors of RT-PCR

With most samples, the MagMAX™-96 AI/ND Viral RNA Isolation Kit protocol yields pure RNA. However, with samples that contain excessively high amounts of reaction inhibitors, sufficient levels of inhibitors can be carried over to inhibit RT-PCR.

Use less RNA in the reaction to minimize the effect of inhibitors

The effect of inhibitors can be minimized or eliminated by reducing the amount of RNA used in qRT-PCR. Try diluting the eluted nucleic acid 10-fold and repeating the RT-PCR. A signal observed using the diluted sample indicates the presence of inhibitors in the eluted nucleic acid.

Detect protein contamination

The UV absorbance at 260 nm and 280 nm can be used to determine if there is protein contamination in the sample. Proteins have an absorbance peak at 280 nm, whereas nucleic acids have an absorbance peak at 260 nm. The ratio of A_{260}/A_{280} is 2.0 for pure nucleic acid that is isolated from cell-free samples with the MagMAX™-96 AI/ND Viral RNA Isolation Kit. An A_{260}/A_{280} ratio below is indicative of protein carryover.

Poor RNA recovery

Evaluate recovery of the Carrier RNA

Using the MagMAX™-96 AI/ND Viral RNA Isolation Kit, 35–75% of the input RNA is expected be recovered (recovery is heavily dependent on sample matrix). Using the recommended volume (101 µL) of prepared Viral Lysis/Binding Solution, each sample contains approximately 1 µg Carrier RNA. Therefore >5 ng/µL is expected to be recovered. Because the Carrier RNA is in great excess relative to viral RNA obtained from the sample, the contribution of viral nucleic acid to the absorbance is negligible.

See “Quantify recovered Carrier RNA“ on page 13 for instructions. Be aware that any cellular DNA or RNA in the prep leads to an overestimation of yield, because all nucleic acids absorb at 260 nm.

Lower-than-expected carrier RNA recovery

Poor recovery of the Carrier RNA could indicate a problem with the viral RNA isolation process. See “Well-to-well variation in RNA yield“ on page 15 for suggestions that can help with RNA recovery. If these suggestions do not improve Carrier RNA recovery, the procedure can require further optimization for use with different sample types. Visit **thermofisher.com/support** for more information on how to optimize the kit for use with various sample types.

Well-to-well variation in RNA yield

The Carrier RNA yield is expected to be uniform between wells of a 96-well plate with the same sample type. However, the efficiency of RNA recovery can differ between different matrices (different types of samples, for example, pharynx/tracheal vs. cloacal swab samples).

RNA binding beads were not fully resuspended

The RNA Binding Beads disperse more easily when the temperature of the mixture is warmer than 20°C.

1. Ensure that the Bead Resuspension Mix is fully resuspended before adding it to the Processing Plate at the start of the procedure.
2. Ensure that the RNA Binding Beads are fully resuspended in Elution Buffer to elute the RNA from the beads. Fully resuspended beads produce a homogeneous brown solution. If the solution is clear, with brown clumps, it means that the beads are not fully resuspended. Preheating the Elution Buffer to 60–65°C just before use helps resuspension of the beads.
3. Avoid overdrying the RNA Binding Beads before eluting the RNA because it can make the beads more difficult to resuspend. If the beads are inadvertently over dried, increase the mixing time to 10 minutes during the elution step to allow the beads to rehydrate.

RNA binding beads were unintentionally lost

Since the principle of this procedure is to immobilize nucleic acids on the RNA Binding Beads, any loss of beads during the procedure will result in loss of RNA. Avoid aspirating the RNA Binding Beads when removing supernatant from the captured beads. To determine whether RNA Binding Beads have been inadvertently aspirated with supernatant, it may be helpful to collect all supernatants (except the final RNA-containing supernatant) in a single container. Observe the color of the collected supernatant. If RNA Binding Beads are in the supernatant, they will color it light brown.

To prevent aspiration of RNA Binding Beads in subsequent experiments, observe the following precautions:

- Use sufficient magnetic capture time.
- Aspirate supernatant slowly.
- Keep pipette tip openings away from the captured RNA Binding Beads and leave ~2 µL liquid behind when aspirating supernatant.

RNA binding bead carryover

If RNA Binding Beads are carried over into the eluate, they cause the solution to be light brown in color. A small quantity of beads in the sample does not inhibit RT reactions or RT-PCR.

- See “RNA binding beads were unintentionally lost“ on page 15 for suggestions to avoid bead carryover.
- To remove RNA Binding Beads from RNA samples, place the Processing Plate on a magnetic stand to capture the beads for 1 minute. Then transfer the nucleic acid solution to a fresh nuclease-free plate or tube.

B

Supplemental information

Isolate the RNA: automated protocol overview

The MagMAX™-96 AI/ND Viral RNA Isolation Kit protocol can be adapted for use with the KingFisher™ Flex Magnetic Particle Processor, MagMAX™ Express-96 Magnetic Particle Processor and KingFisher™ 96 Magnetic Particle Processor (for 1–96 samples per run). The KingFisher™ and MagMAX™ processors completely automate the 12-minute nucleic acid isolation process.

Download instrument protocols at **www.thermofisher.com/us/en/home/life-science/dna-rna-purification-analysis/automated-purification-extraction/automated-protocols-software.html**

1. Pipet the reagents into the indicated row of a 200 µL plate, then insert the plate into the instrument.

Row	Volume	Reagent
A	101 µL	Viral lysis/Binding Solution (Carrier RNA and isopropanol added)
	50 µL	Sample
	20 µL	Bead Resuspension Mix
B	100 µL	Wash Solution 1
C	100 µL	Wash Solution 2
D	100 µL	Wash Solution 2
E	50 µL	Elution Buffer

2. Select the **MagMAX™ AI/ND** program using the arrow keys, then press **Start**.

   The **MagMAX™ AI/ND** program completes the following 12-minute nucleic acid isolation protocol.

   1. Viral RNA is bound to RNA Binding Beads in row A (containing sample, Viral Lysis/Binding Solution, and Bead Resuspension Mix.
   2. The RNA Binding Beads are collected and released into Wash 1 Solution in row B (plate B).
   3. The RNA Binding Beads are collected and released into the first Wash 2 Solution in row C (plate C).
   4. The RNA Binding Beads are collected and released into the second Wash 2 Solution in row D (plate D).
   5. The RNA Binding Beads are collected and lifted outside the wells of row D (plate D) to dry for 1 minute.

6. The RNA Binding Beads are released into Elution Buffer in row E (plate E).
7. The used RNA Binding Beads are collected and returned to row B (plate B) leaving RNA in elution Buffer in row E (plate E).

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

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  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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