MagMAX™-96 AI/ND Viral RNA Isolation Kit

(Cat #AM1835)

Instruction Manual

Manual 1835M Revision D **Revision Date: January 7, 2008**

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I. Introduction

A. Product Description

The MagMAX™-96 AI/ND Viral RNA Isolation Kit (patent pending) 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 and its single-tube, the MagMAX AI/ND Viral RNA Isolation Kit (Cat #AM1929), are 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 #AM1939) and the MagMAX-96 Viral RNA Isolation Kit (Cat #AM1836).

The microspherical paramagnetic beads 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 very consistent yields of high quality RNA with little sample-to-sample variation.

96 samples can be processed at once with the MagMAX-96 AI/ND Viral RNA Isolation Kit; however, it can also be used to efficiently 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 #AM1837) and MagMAX-96 Total RNA Isolation Kit (Cat #AM1830), respectively.

B. Overview of the Procedure

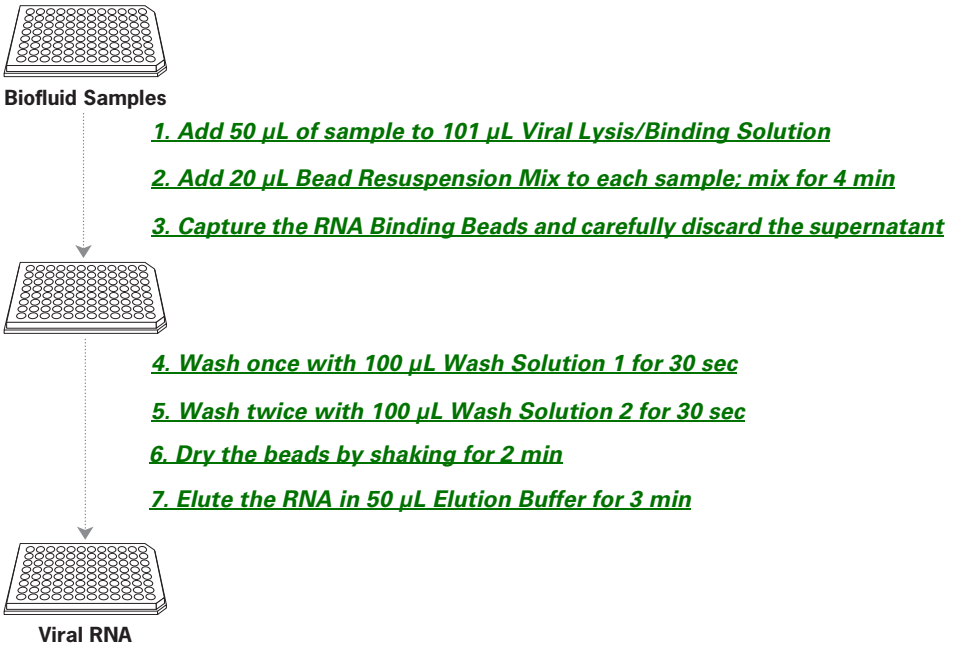
Classic viral particle disruption and magnetic bead-based RNA purification

The MagMAX-96 AI/ND Viral RNA Isolation Kit employs a classic method for disrupting viral particles in a guanidinium thiocyanate-based solution that rapidly releases viral RNA while simultaneous inactivating nucleases in the sample matrix (Chirgwin, et al., 1979; Chomczynski and Sacchi, 1987) (Figure 1). 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 residual binding solutions. RNA is eluted in a small volume of Elution Buffer.

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 upon sample type and is typically greater than 75%. The RNA recovered with the kit is of high

Figure 1. MagMAX-96 AI/ND Viral RNA Isolation Procedure



quality and purity, and is suitable 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.

Manual or robotic high throughput processing

The 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. See Ambion's automation resource page on the web for detailed automation instructions and downloadable protocols for select robotic platforms:

www.ambion.com/techlib/automation

More information on Ambion's high throughput technologies

We are continually working to improve our technologies and expand our line of high throughput products. For the latest updates on our high throughput and automated technologies contact us at:

highthroughput@ambion.com.

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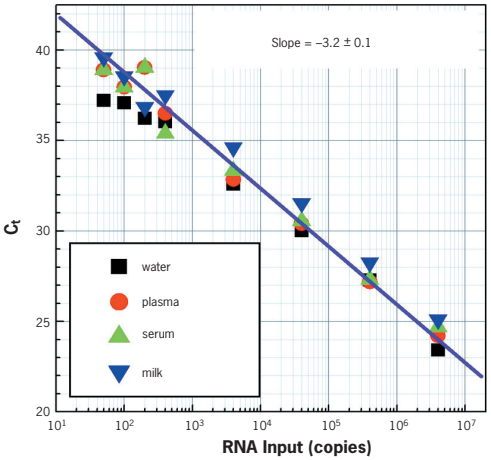

Figure 2. Recovery of Viral RNA Using MagMAX™ -96 AI/ND Viral RNA Isolation Kit.

Serial dilutions of HIV Armored RNA® transcripts were spiked into water, plasma, serum, and milk; and 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.

C. Kit Components and Storage Conditions

The MagMAX-96 AI/ND Viral RNA Isolation Kit contains reagents to isolate RNA from 4 x 96 samples.

Amount	Component	Storage
50 mL	Nuclease-free Water	any temp*
4	Processing Plates and Lids	room temp
50 mL	Viral Lysis/Binding Soln Concentrate (See section II.B.1 on page 6 before use)	room temp†
10 mL	Bead Resuspension Solution (See section II.B.4 on page 7 before use)	room temp†
105 mL	Wash Solution 1 Concentrate (Add 35 mL 100% isopropanol before use)	room temp
100 mL	Wash Solution 2 Concentrate (Add 80 mL 100% ethanol before use)	4°C or room temp
20 mL	Elution Buffer	4°C or room temp
2 mL	RNA Binding Beads (See section II.B.4 on page 7 before use)	4°C†
500 µL	Carrier RNA	–20°C

* Store the Nuclease-free Water at –20°C, 4°C, or room temp.
† Do not freeze these kit components.

D. Required Materials Not Provided With the Kit

Reagents/equipment

- 100% ethanol, ACS grade or higher quality
- 100% isopropanol, ACS grade or higher quality
- To use the kit manually, you will need an orbital shaker for 96-well plates such as the Barnstead/Lab-Line Titer Plate Shaker (VWR #57019-600 or Fisher #14-271-9).
- Magnetic stand for 96-well plates: We recommend the Ambion 96-well Magnetic-Ring Stand (Cat #AM10050) for its high strength magnets and quality design. Most other magnetic stands for 96-well plates may alternatively be used; however, the robotic protocols from Ambion's website would require modification to adjust for stand height and to provide adequate magnetic capture time.
- If you process fewer than 96 samples at a time, you will need additional 96-well U-bottom plates and lids. We recommend polystyrene U-bottom plates and lids from Evergreen Scientific.

Automation equipment

The KingFisher® and KingFisher 96 Magnetic Particle Processors can be used to process samples in about 12 min; a protocol overview is provided in section IV.A on page 15, and a downloadable protocol is available at our automation resource page on the web:

www.ambion.com/techlib/automation

For completely automated RNA isolation with the MagMAX-96 AI/ND Viral RNA Isolation Kit using other systems, the robotic liquid handler must have these features:

- 200 µL pipetting tool
- Gripper tool
- Six reservoirs with reservoir holders
- One magnetic stand for 96-well plates
- Integrated orbital shaker (e.g., MicroMix-5 from Diagnostic Products Corporation).

E. Related Products Available from Ambion

RNase*Zap*® Solution Cat #AM9780, AM9782, AM9784	RNase*Zap* RNase Decontamination Solution is simply sprayed, poured, or wiped onto surfaces to instantly inactivate RNases. Rinsing twice with distilled water will eliminate all traces of RNase and RNase*Zap* Solution.
96-well Magnetic-Ring Stand Cat #AM10050	The Ambion 96-well Magnetic-Ring Stand features 96 powerful ring-shaped magnets arranged to cradle each well of a 96-well plate for quick, thorough bead capture. Captured magnetic beads form evenly distributed donut-shaped pellets with a large hole in the center. This capture pattern facilitates both supernatant removal and subsequent bead resuspension. The stand is suitable for high throughput applications conducted with multichannel pipettors or with robotic liquid handlers. However, because the pellets will be evenly distributed around the edge of the wells, it may require practice for efficient manual removal of supernatants.
Magnetic Stand-96 Cat #AM10027	The Ambion Magnetic Stand-96 has powerful magnets positioned to capture beads to one side of the well. This capture pattern makes it very easy to remove supernatants manually without disturbing the beads, and therefore may be preferred by beginning users. In some applications, however, pellets formed with the Magnetic Stand-96 may be difficult to resuspend. If this occurs, we recommend the 96-well Magnetic-Ring Stand (Cat #AM10050).
MagMAX™ Viral RNA Isolation Kits Cat #AM1929, AM1939	The MagMAX™ and MagMAX AI/ND Viral RNA Isolation Kits are designed for viral RNA and DNA isolation from biological fluids and cell-free samples such as serum, plasma, swabs, and cell culture media. The MagMAX AI/ND Kit (Cat #AM1929) is validated for use in its testing protocol for detection of AI and ND viruses.
MagMAX™-96 Blood RNA Isolation Kit Cat #AM1837	The MagMAX™-96 Blood RNA Isolation Kit is a magnetic bead-based system designed for rapid high throughput isolation of total and viral RNA in 96-well plates from mammalian whole blood and milk. The recovered viral RNA or total RNA can be used directly for quantitative reverse transcriptase PCR (qRT-PCR) for viral veterinary molecular diagnostics or gene expression profiling, respectively.
MagMAX™-96 Total RNA Isolation Kit Cat #AM1830	The MagMAX™-96 Total RNA Isolation Kit is a magnetic bead based total RNA purification system designed for rapid high throughput processing of cells in 96-well plates. High yield and high quality total RNA can be obtained from 100 to 500,000 cultured eukaryotic cells. The kit can also be used for total RNA isolation from small tissue samples.
ArrayScript™ Cat #AM2048, AM2049	ArrayScript is an M-MLV reverse transcriptase engineered to produce high yields of full-length cDNA. In RNA amplification with less than ~100 ng of total RNA, up to twice as much cRNA is synthesized when ArrayScript is used for the RT step compared to wild type M-MLV, AMV, or other engineered M-MLV reverse transcriptases. ArrayScript is also suitable for other RT and RT-PCR applications where high yields of full-length cDNA are required, such as cDNA library construction, 5' RLM-RACE, etc.

II. MagMAX-96 AI/ND Viral RNA Isolation Protocol

A. Equipment Preparation

RNase precautions

Lab bench and pipettors

Before working with RNA, it is always a good idea to clean the lab bench and pipettors with an RNase decontamination solution (e.g., Ambion RNase*Zap*® Solution).

Gloves and RNase-free technique

Wear laboratory gloves for this procedure; they 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.

Determine maximum shaker speed

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

With a Lab-Line orbital shaker, the maximum shaking speed is typically setting 5.5 to 7.5 (there is some variation among individual shakers).

Use this speed for all shaking steps.

(If appropriate) Set up robotic platform and protocol

See Ambion's website automation guidelines, and downloadable protocols for robotic liquid handling systems:
www.ambion.com/techlib/automation

B. Reagent Preparation

1. Add Carrier RNA to the Viral Lysis/Binding Solution Concentrate, mix, then add isopropanol

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.

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.

We do not recommend storing the 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.

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. Store at room temperature.

a. Combine the following:	Per Rxn	1 Plate	Entire Bottle
Viral Lysis/Binding Soln. 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.			

2. Add 35 mL 100% isopropanol to Wash Solution 1 Concentrate

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.

Store at room temperature.

The resulting mixture is called Wash Solution 1 in these instructions.

3. Add 80 mL 100% ethanol to Wash Solution 2 Concentrate

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.

Store at room temperature.

The resulting mixture is called Wash Solution 2 in these instructions.

4. Dilute the Bead Resuspension Solution, add RNA Binding Beads, and add isopropanol

IMPORTANT

Bead Resuspension Mix can be stored at room temp 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.

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.

Store at room temperature.

This mixture is called ***Bead Resuspension Mix*** in these instructions.

a. Combine the following:	Per Rxn	1 Plate	Entire Bottle
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*	4 µL	500 µL	2 mL
c. Mix briefly, then add:			
100% Isopropanol	6 µL	750 µL	3 mL
d. Mix well by vortexing.			

* Mix the RNA Binding Beads thoroughly by vortexing before dispensing.

C. RNA Isolation Protocol

Sample type and volume
Pharynx/tracheal and cloacal swabs collected in BHI or VTM media are the recommended sample type. Use up to 50 µL sample per prep.

Number of samples
96 samples can be processed at once. To process fewer samples at a time additional polystyrene U-bottom 96-well plates and lids will be needed.

Shaking Speed
Use the maximum shaker speed identified in section A. on page 6 for all of the shaking steps in the protocol.

1. Add 50 µL of sample to 101 µL Viral Lysis/Binding Solution

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

b. 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.

c. Shake the plate for 30 sec on an orbital shaker at the speed identified in section A. on page 6.

2. Add 20 µL *Bead Resuspension Mix* to each sample; mix for 4 min

a. Vortex the Bead Resuspension Mix to fully resuspend the beads before pipetting.

b. Add 20 µL Bead Resuspension Mix to each sample (prepared as described in step B. on page 7).

c. Shake the plate for 4 min to fully lyse viruses and bind RNA to the RNA Binding Beads.

3. Capture the RNA Binding Beads and carefully discard the supernatant

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

b. Carefully aspirate and discard the supernatant without disturbing the beads.

IMPORTANT

To obtain pure RNA, it is important to completely remove the supernatant at this step. Use the Ambion 96-well Magnetic-Ring Stand (Cat #AM10050) for the best consistency.

4. Wash once with 100 µL Wash Solution 1 for 30 sec

a. Remove the Processing Plate from the magnetic stand.

b. Add 100 µL Wash Solution 1 (isopropanol added) to each sample and shake the plate for 30 sec.
The RNA Binding Beads may not fully disperse during this step; this is expected, and it will not effect RNA purity or yield.

c. Capture the RNA Binding Beads on a magnetic stand for ~1 min, or until the mixture becomes clear.

d. Carefully aspirate and discard the supernatant without disturbing the beads.

5. Wash twice with 100 µL Wash Solution 2 for 30 sec

a. Remove the Processing Plate from the magnetic stand.

b. Add 100 µL Wash Solution 2 (ethanol added) to each sample and shake the plate at moderate speed for 30 sec.

c. Capture the RNA Binding Beads as in the previous wash.

d. Carefully aspirate and discard the supernatant without disturbing the beads.

e. Repeat steps 5.b–d to wash with a second 100 µL of Wash Solution 2.

IMPORTANT

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

6. Dry the beads by shaking for 2 min

a. Inspect the wells of the Processing Plate, and if there is residual 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.

b. Move the Processing Plate to the shaker and shake vigorously for 2 min to allow any remaining alcohol from the Wash Solution 2 to evaporate.


IMPORTANT

Do not shake the plate for >5 min, as this could overdry the beads.

7. Elute the RNA in 50 µL Elution Buffer for 3 min

a. Add 50 µL Elution Buffer (room temp or prewarmed to 37–65°C) to each sample, and shake 3 min.


NOTE

The RNA can be eluted as little as 20 µL. The volume of Elution Buffer supplied with the kit is enough for 4 x 96 samples at 50 µL each.

b. Capture the RNA Binding Beads as in the previous steps. ***The purified RNA will be in the supernatant.***

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


NOTE

Leaving 1–2 µL liquid behind will minimize bead carryover into the storage plate.

d. Store the purified RNA at –20°C.

D. Analyzing Viral RNA

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.

Quantitate Carrier RNA recovered

The viral RNA recovered from most samples will be present in very limited amounts; the majority of RNA in the purified sample will be the Carrier RNA that was added to the Viral Lysis/Binding Solution. RNA recovery is heavily dependent upon sample type (e.g., plasma vs. swab samples). With most sample types, up to 75% of the carrier RNA should be recovered. Using the recommended volume (101 µL) of prepared Lysis/Binding Solution, each sample will contain approximately 1 µg Carrier RNA; therefore >5 ng/µL RNA should be recovered.

Quantitate the amount of Carrier RNA by UV absorbance at 260 nm (A_{260}). Ambion scientists recommend the NanoDrop® 1000A Spectrophotometer (www.nanoambion.com). Absorbance readings using the NanoDrop are quick 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} X dilution factor X 40 = µg RNA/mL

III. Troubleshooting

A. 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 protocol yields very pure RNA; however, with samples that contain excessively high amounts of reaction inhibitors, enough may be carried over to inhibit RT-PCR.

Minimize the effect of inhibitors by using less RNA in the reaction

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; if a signal is observed using the diluted sample, this would indicate the presence of inhibitors in the eluted nucleic acid.

Detecting protein contamination

The UV absorbance at 260 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} should be ~2.0 for pure nucleic acid isolated from cell-free samples with the MagMAX-96 AI/ND Viral RNA Isolation Kit. An A_{260}/A_{280} ratio below this is indicative of protein carryover.

Poor RNA recovery

Evaluate recovery of the Carrier RNA

Using the MagMAX-96 AI/ND Viral RNA Isolation protocol, 35–75% of the input RNA should be recovered (recovery is heavily dependent upon sample matrix). Using the recommended volume (101 µL) of prepared Viral Lysis/Binding Solution, each sample will contain approximately 1 µg Carrier RNA; therefore >5 ng/µL should be recovered. Since 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.

Instructions for quantitating Carrier RNA are found in section *II.D. Quantitate Carrier RNA recovered* on page 10. Be aware that any cellular DNA or RNA in the prep will lead to an overestimation of yield, since 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 section *B. Well-to-Well Variation in RNA Yield* below for suggestions that may help with RNA recovery. If these suggestions do not improve Carrier RNA recovery, the procedure may require further optimization for use with different sample types; contact Ambion's Technical Services Department for more information on how to optimize the kit for use with various sample types.

B. Well-to-Well Variation in RNA Yield

The Carrier RNA yield should be fairly uniform between wells of a 96-well plate with the same sample type. However, the efficiency of RNA recovery may differ between different matrices (different types of samples, e.g., pharynx/tracheal vs. cloacal swab samples). If large variations in nucleic acid yield are observed, consider the following suggestions:

RNA Binding Beads were not fully resuspended/dispersed

In general, the RNA Binding Beads will disperse more easily when the temperature of the mixture is warmer than 20°C.

a. Make sure the Bead Resuspension Mix is fully resuspended before adding it to the Processing Plate at the start of the procedure (step II.C.2 on page 8).

b. Make sure that the RNA Binding Beads are fully resuspended in Elution Buffer to efficiently elute the RNA from the beads in step II.C.7 on page 10. Fully resuspended beads will produce a homogenize 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 will facilitate resuspension of the beads.

c. Avoid overdrying the RNA Binding Beads before eluting the RNA (in step II.C.b on page 9) because this may make the beads more difficult to resuspend. If the beads are inadvertently overdried, increase the mixing time (to 10 min) during the elution step (II.C.7) 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.

C. RNA Binding Bead Carryover

If RNA Binding Beads are carried over into the eluate, they will 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 section *III.B. RNA Binding Beads were unintentionally lost* on page 13 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 min. Then transfer the nucleic acid solution(s) to a fresh nuclease-free plate or tubes.

IV. Appendix

A. KingFisher MagMAX-96 AI/ND Viral RNA Isolation Protocol Overview

The MagMAX-96 AI/ND Viral RNA Isolation protocol can be adapted for use with Thermo Electron's KingFisher (for 1–24 samples per run) and KingFisher 96 (for 1–96 samples per run) Magnetic Particle Processors. Downloadable protocols for these machines are available on Ambion's automation resource page on the web:

www.ambion.com/techlib/automation

The KingFisher processors completely automate the nucleic acid isolation process; here is a quick overview of how it works:

1. Pipet MagMAX-96 AI/ND Viral RNA Isolation Kit reagents into a KingFisher 200 µL plate(s) and insert the plate(s) into the KingFisher or KingFisher 96 instrument.

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

2. Choose the MagMAX AI/ND program using the arrow keys and start the program by pressing the START button. The approximately 12 min process is described below:
3. Viral RNA is bound to RNA Binding Beads in row A (plate A the KingFisher 96) containing sample, Viral Lysis/Binding Solution, and Bead Resuspension Mix.
4. The RNA Binding Beads are collected and released into Wash 1 Solution in row B (plate B).
5. The RNA Binding Beads are collected and released into the first Wash 2 Solution in row C (plate C).
6. The RNA Binding Beads are collected and released into the second Wash 2 Solution in row D (plate D).
7. The RNA Binding Beads are collected and lifted outside the wells of row D (plate D) to dry for 1 min.

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

B. References

Chirgwin J, Przybyla A, MacDonald A, and Rutter W (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochem.* **18**:5294.

Chomczynski P and Sacchi N (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analyt. Biochem.* **162**:156–159.

C. Safety Information

The MSDS for any chemical supplied by Applied Biosystems or Ambion is available to you free 24 hours a day.

IMPORTANT

For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.

To obtain Material Safety Data Sheets

- Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: www.ambion.com/techlib/msds
- Alternatively, e-mail your request to MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery.
- For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.

- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

D. Quality Control

Functional testing

All kit components are tested functionally by isolating the carrier RNA and an Armored RNA using the protocol described in this manual. RNA recovery is assessed by absorbance measurements using the NanoDrop Spectrophotometer and by qRT-PCR. RNA integrity is evaluated using an Agilent® 2100 bioanalyzer.

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

RNase activity

Meets or exceeds specification when a sample is incubated with 25 ng labeled RNA and analyzed by PAGE.

Nonspecific endonuclease activity

Meets or exceeds specification when a sample is incubated with 300 ng supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity

Meets or exceeds specification when a sample is incubated with 40 ng labeled *Sau*3A fragments of pUC19 and analyzed by PAGE.