MagMAX™ AI/ND Viral RNA Isolation Kit

(Cat #AM1929)

Instruction Manual

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I. Introduction

A. Product Description

The MagMAX™ AI/ND Viral RNA Isolation Kit (patent pending) is designed for rapid purification of Avian Influenza (AI) and/or Newcastle Disease (ND) viral RNA from pharynx/tracheal and cloacal swab samples. This kit and its high throughput counterpart, the MagMAX-96 AI/ND Viral RNA Isolation Kit (Cat #AM1835), 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.

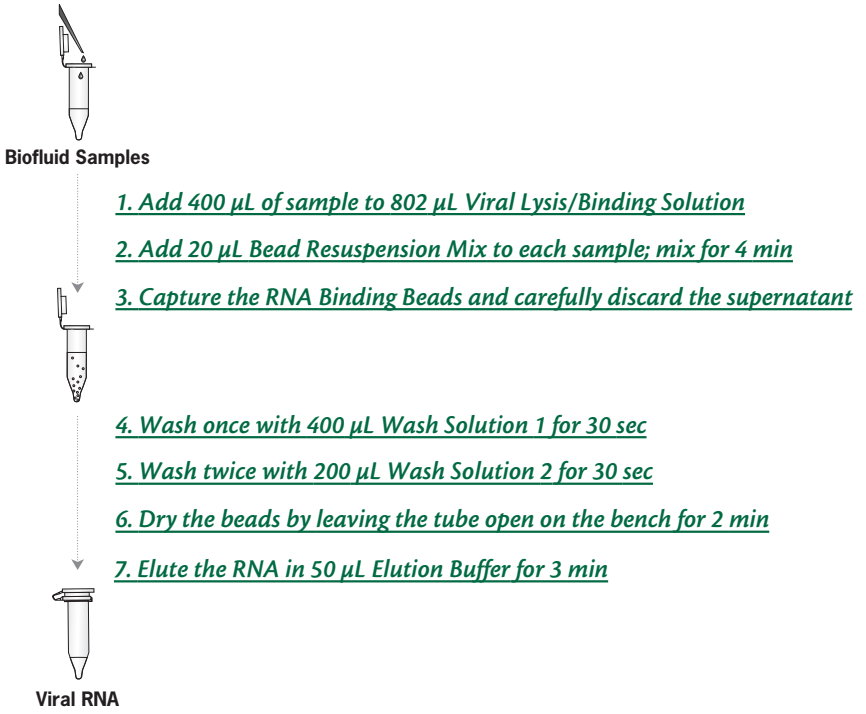
The procedure in this Instruction Manual is written primarily for low throughput microcentrifuge tube based viral RNA isolation. However, this kit can be used in a 96-well format as a trial version of the high throughput kit by preparing reagents as described in section IV.A on page 15 in this booklet and following the protocol in the MagMAX-96 AI/ND Viral RNA Isolation Kit Instruction Manual (Cat #AM1835), available on our website at: www.ambion.com/catalog/CatNum.php?1835. 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 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.

Figure 1. MagMAX AI/ND Viral RNA Isolation Procedure



NOTE

This protocol is appropriate for isolating viral RNA in microcentrifuge tube format. To use the kit in 96-well plate format, use the reagent preparation instructions in section IV.A on page 15 of this booklet and follow the protocol in the MagMAX-96 AI/ND Viral RNA Isolation Kit Instruction Manual (Cat #1835), available on our website at: www.ambion.com/catalog/CatNum.php?1835.

Sample size and RNA recovery

The MagMAX AI/ND Viral RNA Isolation Kit can efficiently isolate viral RNA from biofluid samples as large as 400 µL containing as few as 10 RNA copies. RNA recovery is dependant upon sample type and is typically greater than 75%. The RNA recovered with the kit is of high 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.

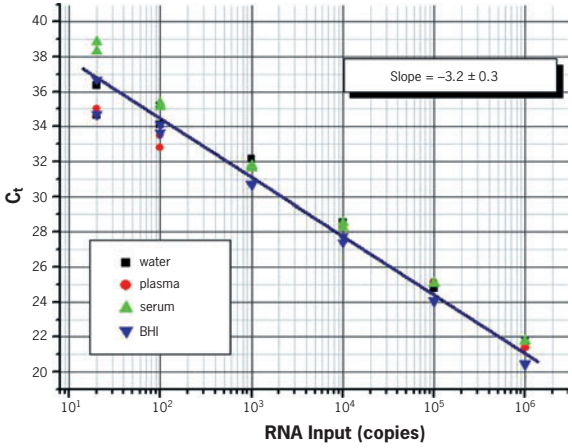


Figure 2. Recovery of Viral RNA Using the MagMAX™ AI/ND Viral RNA Isolation Kit.

Serial dilutions of HIV Armored RNA® transcripts were spiked into water, plasma, serum, and BHI (brain heart infusion broth) and viral RNA was isolated using the MagMAX AI/ND Viral RNA Isolation Kit according to the protocol. Equivalent volumes of recovered viral RNA (1/4 of eluted volume) were used in a 25 µL qRT-PCR to detect the HIV transcript.

* This product is compatible with the 5' nuclease detection and dsDNA-binding dye processes covered by patents owned or licensable by Applied Biosystems. No license under these patents is conveyed expressly, by implication, or by estoppel to the purchaser by the purchase of this product. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

C. Kit Components and Storage Conditions

The MagMAX AI/ND Viral RNA Isolation Kit contains reagents to isolate RNA from 50 samples.

Amount	Component	Storage
5 mL	Nuclease-free Water	any temp*
50	Processing Tubes	room temp
44 mL	Viral Lysis/Binding Soln Concentrate (See section II.A.2 on page 6 before use)	room temp†
1.1 mL	Bead Resuspension Solution (See section II.A.5 on page 7 before use)	room temp†
24 mL	Wash Solution 1 Concentrate (Add 8 mL 100% isopropanol before use)	room temp
25 mL	Wash Solution 2 Concentrate (Add 20 mL 100% ethanol before use)	4°C or room temp
5 mL	Elution Buffer	4°C or room temp
550 µL	RNA Binding Beads (See section II.A.5 on page 7 before use)	4°C†
110 µL	Carrier RNA	–20°C

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

Properly stored kits are guaranteed for 6 months from the date received.

D. Required Materials Not Provided With the Kit

Reagents

- 100% ethanol, ACS grade or higher quality
- 100% isopropanol, ACS grade or higher quality

Equipment

- A vortex mixer (optional: a vortex adapter for hands-free mixing of several samples simultaneously, e.g. Ambion Cat #AM10024).
- Magnetic stand for 1.5 mL tubes, e.g. the Ambion 6 Tube Magnetic Stand (Cat #AM10055)

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.
6 Tube Magnetic Stand Cat #AM10055	Designed to hold up to six standard microcentrifuge tubes, the 6 Tube Magnetic Stand houses a strong magnet for fast, efficient separation of magnetic beads from solutions.
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™-96 Viral RNA Isolation Kits Cat #AM1835, AM1836	The MagMAX™-96 and MagMAX-96 AI/ND Viral RNA Isolation Kits are designed for rapid high throughput purification of viral RNA and DNA in 96 well plates from biological fluids and cell-free samples such as serum, plasma, swabs, and cell culture media. The MagMAX-96 AI/ND Kit (Cat #AM1835) is validated for use in its U.S. National Veterinary Service Laboratories testing protocol for detection of AI and ND viruses.
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.
Vortex Adapter Cat #AM10024	The Vortex Adapter attaches to Vortex-Genie® 2 (Scientific Industries) vortex mixers to hold microfuge tube for hands-free agitation.

II. MagMAX AI/ND Viral RNA Isolation Protocol

A. Equipment and Reagent Preparation

1. 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.

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

IMPORTANT

For viral RNA isolation in a 96-well format, follow the modified procedure for preparation of Viral Lysis Binding Solution described in section IV.A on page 15.

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	10 Rxns	Entire Bottle
Viral Lysis/Binding Soln. Concentrate	400 µL	4.4 mL	22 mL
Carrier RNA	2 µL	22 µL	110 µL
b. Mix briefly, then add:			
100% Isopropanol	400 µL	4.4 mL	22 mL
c. Mix well by vortexing.			

3. Add 8 mL 100% isopropanol to Wash Solution 1 Concentrate

Add 8 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.

4. Add 20 mL 100% ethanol to Wash Solution 2 Concentrate

Add 20 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.

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

IMPORTANT

For viral RNA isolation in a 96-well format, follow the modified procedure for preparation of Bead Resuspension Mix described in section IV.A on page 15.

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.

Combine Bead Resuspension Solution with RNA Binding Beads according to the table below, and mix briefly. Then 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	10 Rxns	Entire Bottle
RNA Binding Beads*	10 µL	110 µL	550 µL
Bead Resuspension Solution	4 µL	44 µL	220 µL
b. Mix briefly, then add:			
100% Isopropanol	6 µL	66 µL	330 µL
c. Mix well by vortexing.			

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

B. RNA Isolation Protocol

Sample type

Pharynx/tracheal and cloacal swabs collected in BHI or VTM media are the recommended sample type.

Sample Volume

With up to 400 µL sample input, the MagMAX AI/ND Viral RNA Isolation procedure can be completed in the 1.5 mL tubes provided in the kit.

IMPORTANT

It is possible to process 50 µL samples in 96-well format by using Viral Lysis/Binding Solution and Bead Resuspension Mix prepared as described in section IV.A on page 15 of this booklet and following the "RNA Isolation Protocol" in the MagMAX-96 AI/ND Viral RNA Isolation Instruction Manual available on our website at: www.ambion.com/catalog/CatNum.php?1835.

1. Add 400 µL of sample to 802 µL Viral Lysis/Binding Solution

a. Add 802 µL prepared Viral Lysis/Binding Solution (Carrier RNA and isopropanol added) to each Processing Tube.

b. Transfer up to 400 µL of sample to each Processing Tube 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. Mix by gently vortexing for 30 sec. Then centrifuge briefly (~2 sec) to collect the contents at the bottom of the tube.

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 A. on page 7).

c. Gently vortex the sample for 4 min to fully lyse viruses and bind RNA to the RNA Binding Beads.

d. Centrifuge briefly (~2 sec) to collect the tube contents.

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

a. Move the Processing Tube to a magnetic stand to capture the RNA Binding Beads. Leave the tube on the magnetic stand for 3 min, or until the mixture becomes clear. When capture is complete, the RNA Binding Beads will form a pellet against the magnet 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.

4. Wash once with 400 µL Wash Solution 1 for 30 sec

a. Remove the Processing Tube from the magnetic stand.

b. Add 400 µL Wash Solution 1 (isopropanol added) to each sample and vortex at moderate speed 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. Centrifuge briefly (~2 sec) to collect tube contents.

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

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

5. Wash twice with 200 µL Wash Solution 2 for 30 sec

a. Remove the Processing Tube from the magnetic stand.

b. Add 200 µL Wash Solution 2 (ethanol added) to each sample and vortex at moderate speed for 30 sec.

c. Centrifuge briefly (~2 sec) to collect tube contents.

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

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

f. Repeat steps 5.b–e to wash with a second 200 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 leaving the tube open on the bench for 2 min

a. Leave the tube open at room temp for 2 min to allow any remaining alcohol from the Wash Solution 2 to evaporate.

b. Inspect the tubes, and if there is residual solution, remove as much as possible with a fine-tipped pipette. Then leave the tube open on bench for another minute.

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

a. Remove the Processing Tube from the magnetic stand.

b. Add 50 µL Elution Buffer (room temp or prewarmed to 37–65°C) to each sample, and vortex vigorously for3 min.

NOTE

The RNA can be eluted as little as 20 µL. The volume of Elution Buffer supplied with the kit is enough for 50 samples at 100 µL each.

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

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

e. Store the purified RNA at –20°C.

C. 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 (802 µL) of prepared Lysis/Binding Solution, each sample will contain approximately 2 µg of Carrier RNA; therefore >10 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 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 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 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 (802 µL) of prepared Viral Lysis/Binding Solution, each sample will contain approximately 2 µg of Carrier RNA; therefore >10 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.C. 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. Sample-to-Sample 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. Sample-to-Sample Variation in RNA Yield

The Carrier RNA yield should be fairly uniform among samples 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 Tube at the start of the procedure (step II.B.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.B.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.B.a on page 10) 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.B.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 Tube 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. Reagent Preparation for Use of the Kit in 96-well Format

Preparation of Viral Lysis/Binding Solution

a. Combine the following:	Per Rxn	~100 Rxns
Viral Lysis/Binding Soln. Concentrate	50 µL	5.5 mL
Carrier RNA	1 µL	110 µL
b. Mix briefly, then add:		
100% Isopropanol	50 µL	5.5 mL
c. Mix well by vortexing.		

Preparation of Bead Resuspension Mix

a. Combine the following:	Per Rxn	~100 Rxns
Bead Resuspension Solution	6 µL	660 µL
Nuclease-free water	4 µL	440 µL
b. Mix briefly, then add:		
RNA Binding Beads*	4 µL	440 µL
c. Mix briefly, then add:		
100% Isopropanol	6 µL	660 µL
d. Mix well by vortexing.		

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

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. MagMAX AI/ND Viral RNA Isolation Kit Specifications

Kit contents and storage conditions:

The MagMAX AI/ND Viral RNA Isolation Kit contains reagents to isolate RNA from 50 samples.

Amount	Component	Storage
5 mL	Nuclease-free Water	any temp*
50	Processing Tubes	room temp
44 mL	Viral Lysis/Binding Soln Concentrate	room temp†
1.1 mL	Bead Resuspension Solution	room temp†
24 mL	Wash Solution 1 Concentrate	room temp
25 mL	Wash Solution 2 Concentrate	4°C or room temp
5 mL	Elution Buffer	4°C or room temp
550 µL	RNA Binding Beads	4°C†
110 µL	Carrier RNA	–20°C

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

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.)

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.