USER GUIDE

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MagMAX™-96 Viral RNA Isolation Kit

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For Veterinary Use

life technologies™

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MagMAX™-96 Viral RNA Isolation Kit

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product information

Product description

The MagMAX™-96 Viral RNA Isolation Kit is designed for rapid high throughput purification of viral RNA and DNA from biofluid samples such as serum, plasma, nasal fluid, and swab samples in 96-well plates. 96 samples can be processed at once with the MagMAX™-96 Viral RNA Isolation Kit; however, it can also be used to efficiently isolate RNA and DNA from fewer than 96 samples. For viral nucleic acid isolation from whole blood, semen or oral fluids, we recommend the MagMAX™ Pathogen DNA/RNA Kit. For tissue samples, we recommend the MagMAX™-96 Total RNA Isolation Kit.

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

Kit components and storage conditions

The MagMAX™-96 Viral RNA Isolation Kit contains reagents to isolate RNA from 96 samples.

Amount	Component	Storage
1	Processing Plate and Lid	room temp
16 mL	Lysis/Binding Solution Concentrate See "Reagent preparation" on page 8 before use	room temp
36 mL	Wash Solution 1 Concentrate (Add 12 mL of 100% isopropanol before use)	room temp
40 mL	Wash Solution 2 Concentrate (Add 32 mL of 100% ethanol before use)	room temp
9 mL	Elution Buffer	room temp
1.1 mL	RNA Binding Beads	4°C†
125 µL	Carrier RNA	–20°C
1.1 mL	Lysis/Binding Enhancer	–20°C

† Do not freeze the RNA Binding Beads.

Required materials not provided with the kit

Reagents/equipment

- 100% ethanol, ACS grade or better
- 100% isopropanol, ACS grade or better
- 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 96-well Magnetic-Ring Stand (Cat. no. 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 for the MagMAX™ Express-96 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 MagMAX™ Express (Cat. no. 4400074) and MagMAX™ Express-96 Standard (Cat. no. 4400076) Magnetic Particle Processors can be used to process samples in about 25 min. The MagMAX™ Express-96 instrument can be equipped with a Deep Well Magnetic Head (Cat. no. 4388435). Downloadable protocols are available on our website at the following address:

www.lifetechnologies.com/magmaxexpress/scripts

Other materials required:

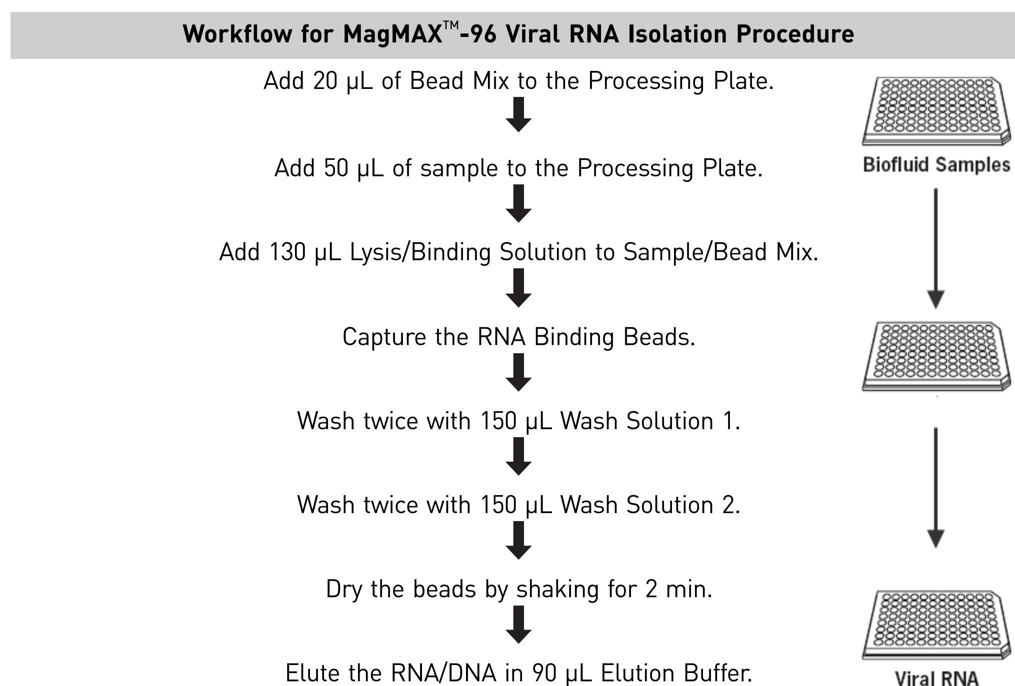
Instrument	Item	Cat. no.
MagMAX™ Express	MagMAX™ Express Plates	4388474
	MagMAX™ Express Tip Combs	4388452
MagMAX™ Express-96 Standard Well config.	MagMAX™ Express-96 Standard Well Plates	4388475
	MagMAX™ Express-96 Standard Well Tip Combs	4388488
MagMAX™ Express-96 Deep Well config.	MagMAX™ Express-96 Deep Well Plates	4388476
	MagMAX™ Express-96 Deep Well Tip Combs	4388487

Overview of the procedure

The MagMAX™-96 Viral RNA Isolation Kit employs a classic method for disrupting samples in a guanidine isothiocyanate-based solution that rapidly releases viral RNA and DNA while simultaneously inactivating nucleases in the sample matrix (Chirgwin, et al., 1979; Chomczynski and Sacchi, 1987). 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 solution. Nucleic acids are eluted in a small volume of elution buffer. Note that this procedure recovers total nucleic acids, so if cells are present in the sample, cellular DNA/RNA will be recovered along with the viral RNA.

The MagMAX™-96 Viral RNA Isolation Kit can efficiently isolate viral RNA and DNA from 50 µL of sample. 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†.

Workflow for MagMAX™-96 Viral RNA Isolation Procedure



Manual or robotic high throughput processing

The MagMAX™-96 Viral RNA Isolation Kit is optimized both for robotic liquid handlers and for use manually with multichannel pipettors. Visit this resource page for detailed automation instructions and downloadable protocols for select robotic platforms:

www.lifetechnologies.com/magmaxexpress/scripts

† This product is compatible with the 5' nuclease detection and dsDNA-binding dye processes covered by patents owned or licensable by Applied Biosystems by Life Technologies. 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 outlicensing@lifetechnologies.com.

Methods

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 ~210 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 of the shaking incubations in the protocol.

Reagent preparation

Stepwise procedure:

1. **Add carrier RNA to the 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 the tube of Carrier RNA at 37°C for 10–15 min.

Prepared Lysis/Binding Solution is stable at room temperature for one month. We recommend preparing only enough Lysis/Binding Solution for one day by scaling down the reagent volumes proportionally. We do not recommend storing the prepared Lysis/Binding Solution at 4°C or below as this may cause the solution to form a 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 Lysis/Binding Solution Concentrate according to the table below, and mix briefly. Then add 100% isopropanol and mix well. This mixture is called Lysis/Binding Solution in these instructions.

We recommend including ~10% overage to cover pipetting error when preparing the Lysis/Binding Solution. If you prepare the entire bottle, mark the label to indicate that the Carrier RNA and isopropanol were added. Store at room temperature.

Note: If you have an internal positive control RNA to monitor viral RNA purification and detection, we recommend adding it to the Lysis/Binding Concentrate before adding the isopropanol.

a. Combine the following:	Per Reaction	Whole Bottle
Lysis/Binding Solution Concentrate	65 µL	8 mL
Carrier RNA (1 µg/rxn)	1 µL	125 µL
Optional: Internal Positive Control Nucleic Acid	1 µL	-
b. Mix briefly, then add:		
100% isopropanol	65 µL	8 mL
c. Mix well by vortexing		

2. **Add 12 mL 100% isopropanol to Wash Solution 1 Concentrate.**

   Add 12 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 32 mL 100% ethanol to Wash Solution 2 Concentrate.**

   Add 32 mL 100% ethanol to the bottle labeled as 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. Prepare *Bead Mix*.

   Each isolation reaction requires 20 µL of Bead Mix. Although the mixture is stable at 4°C for up to 2 weeks, we recommend preparing ***Bead Mix*** on the day it will be used.

   a. Vortex the RNA Binding Beads at moderate speed to form a uniform suspension before pipetting.

   b. Prepare Bead Mix by combining the volumes of RNA Binding Beads and Lysis/Binding Enhancer shown in the table below appropriate for the number of isolation reactions to be performed that day. Mix thoroughly by gently vortexing. We recommend including ~10% overage to cover pipetting error when preparing the Bead Mix.

Component	Per Reaction	96 Rxns (+~10%)
RNA Binding Beads	10 µL	1.1 mL
Lysis/Binding Enhancer	10 µL	1.1 mL

   c. Place the prepared Bead Mix on ice until it is needed in step 2 on page 10.

RNA isolation procedure

Sample type

This kit is designed for isolation of viral RNA and DNA from cell-free, or nearly cell-free samples. For example, biological fluids such as serum, plasma, urine, meconium, and nasal fluids can be used with the kit. Other common sample types such as cell culture medium and swab samples are also compatible.

Sample volume

With up to 50 µL sample input, the MagMAX™-96 Viral RNA Isolation procedure can be completed in the U-bottom 96-well plate provided in the kit. 50 µL of sample is adequate for most applications; however, larger volumes can be processed (e.g., to increase the detection sensitivity of low titer samples) using the MagMAX™ Pathogen RNA/DNA Isolation Kit (Cat. no. 4462359), which can accommodate up to 300 µL of sample input. Please contact Life Technologies at Animalhealth@lifetech.com for recommendations on your specific applications.

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.

Stepwise procedure:

1. Add 20 µL of Bead Mix to the Processing Plate.
   a. Vortex the Bead Mix at moderate speed to create a uniform suspension before pipetting (prepared as described in step 4 on page 9).
   b. Pipet 20 µL Bead Mix into each well of the Processing Plate.
2. Add 50 µL of sample to the Processing Plate.
   a. Add 50 µL of sample to wells in the Processing Plate.

      Note: When adding sample, immerse pipette tips slightly in the Bead Mix to prevent creating aerosols that can lead to cross-contamination.
   b. Shake the plate for 1 min on an orbital shaker at the maximal speed identified on page 8.
3. Add 130 µL Lysis/Binding Solution to Sample/Bead Mix.
   a. Add 130 µL of the Lysis/Binding Solution (Carrier RNA and isopropanol added, as described in step 1 on page 8) into wells of the Processing Plate containing Sample/Bead Mix.
   b. Shake the plate for 5 min on an orbital shaker at the maximal speed.

      Note: If you are working with viruses that are known to be difficult to lyse, extend this shaking incubation to 10 min to improve lysing efficiency.
4. Capture the RNA Binding Beads.
   a. Move the Processing Plate to a magnetic stand to capture the RNA Binding Beads. Leave the plate on the magnetic stand for at least 3 min. 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. Remove the Processing Plate from the magnetic stand.

      IMPORTANT! To obtain pure RNA, it is important to completely remove the supernatant at this step. Use the 96-well Magnetic-Ring Stand (Cat. no. AM10050) for the best consistency.

5. Wash twice with 150 µL Wash Solution 1.
   a. Add 150 µL Wash Solution 1 (isopropanol added) to each sample and shake the plate for 1 min.
   b. Capture the RNA Binding Beads on a magnetic stand for ~1 min, or until the mixture becomes clear, indicating that capture is complete.
   c. Carefully aspirate and discard the supernatant without disturbing the beads. Remove the Processing Plate from the magnetic stand.
   d. Repeat steps 5a–c to wash with a second 150 µL of Wash Solution 1. It is critical to move the Processing Plate off the magnetic stand before the next wash.
6. Wash twice with 150 µL Wash Solution 2.
   a. Add 150 µL Wash Solution 2 (ethanol added) to each sample and shake for 1 min.
   b. Capture the RNA Binding Beads as in the previous wash.
   c. Carefully aspirate and discard the supernatant without disturbing the beads and remove the Processing Plate from the magnetic stand. As in the previous wash, it is critical to move the Processing Plate off the magnetic stand before the subsequent wash.
   d. Repeat steps 6a–c to wash with a second 150 µL of Wash Solution 2. It is important to remove as much Wash Solution 2 from the samples as possible, because otherwise it could inhibit downstream applications such as RT-PCR.

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

7. Dry the beads by shaking for 2 min.
   a. Move the Processing Plate to the shaker and shake for 2 min without the Processing Plate lid to allow any remaining alcohol from the Wash Solution 2 to evaporate.
   b. Inspect the wells and if there is residual solution, shake the plate for another minute or two to let it evaporate.

   IMPORTANT! Do not shake the plate for more than 5 min, as this could overdry the beads and make it difficult to elute the RNA/DNA from the beads.

8. Elute the RNA/DNA in 90 µL Elution Buffer.
   a. Add 90 µL Elution Buffer (room temp or pre-warmed to 60–65°C; see Troubleshooting "RNA Binding Beads were not fully resuspended/ dispersed" on page 13) to each sample, and shake vigorously for 3 min.

      Note: The elution volume is somewhat flexible; RNA/DNA can be eluted as little as 20 µL, or up to 90 µL to achieve the desired final nucleic acid concentration. The volume of Elution Buffer supplied with the kit is enough for 96 samples at 90 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, and store the purified RNA at –20°C.

Analyzing viral RNA and DNA

Detect viral RNA by real-time RT-PCR and detect DNA by real-time PCR

This kit is designed for purification of RNA for RT-PCR amplification, and purification of DNA for qPCR. Quantitative real-time RT-PCR/PCR is a powerful method for viral RNA/DNA detection and is the recommended analysis tool.

Quantify 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 Lysis/Binding Solution. RNA recovery is heavily dependent upon sample type (e.g., plasma vs. swab samples). With most sample types, 35-75% of the carrier RNA should be recovered. Using the recommended volume (130 µL) of prepared Lysis/Binding Solution, each sample will contain approximately 1 µg Carrier RNA; therefore ~5 ng/µL RNA should be recovered.

Quantify the amount of Carrier RNA by UV absorbance at 260 nm (A_{260}). If you are using a NanoDrop® 1000 Spectrophotometer, 1.5 µL of nucleic acid solution can be measured without dilution.

Alternatively, the RNA concentration can be determined by diluting an aliquot of the preparation in TE buffer (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

Troubleshooting

Poor viral nucleic acid detection

If poor or no viral RNA or DNA signal is observed by RT-PCR (i.e., the Ct is later 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 Viral RNA Isolation procedure yields very pure nucleic acid; however, with samples that contain excessively high amounts of reaction inhibitors, enough may be carried over to affect RT-PCR.

Minimize the effect of inhibitors by adding a lower volume of eluted nucleic acid to the reaction. The effect of inhibitors can be limited by diluting the eluted nucleic acid thereby diluting out the inhibitor. Try diluting the eluted nucleic acid 10-fold and repeating the RT-PCR or PCR; if a signal is observed using the diluted sample, this would indicate the presence of inhibitors in the eluted nucleic acid. The use of an internal positive control that is included in the extraction can be used to monitor inhibition.

Poor RNA or DNA recovery: assess recovery of the carrier RNA

- **Expected Carrier RNA recovery.** Using the MagMAX™-96 Viral RNA Isolation procedure, 35–75% of the input RNA should be recovered (recovery is heavily dependent upon sample type). Using the recommended volume (130 µL) of prepared Lysis/Binding Solution, each sample will contain approximately 1 µg Carrier RNA; therefore ~5 ng/µL RNA should be recovered. Instructions for quantitating Carrier RNA recovery are found on page 12. 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.
- **Good recovery of Carrier RNA, but viral RNA or DNA cannot be detected.** If the Carrier RNA was recovered at expected levels (~5 ng/µL RNA), but viral nucleic acid cannot be detected using a proven qRT-PCR or qPCR assay system, this would suggest the absence of virus in the original sample, poor lysis of viral particles, or problems with the RT-PCR or PCR. Consider the recommendation in the previous section for diluting your sample to minimize the effects of inhibitors. In addition, improper sample handling can also lead to poor results. Freezing and thawing samples multiple times can lead to nucleic acid degradation.

  Troubleshoot viral particle lysis: If diluting the viral nucleic acid does not help, the problem is most likely due to incomplete lysis of viral particles. Consider increasing the incubation time of the lysis/binding in step 3 on page 10 to 10–15 min.
- **Lower-than-expected Carrier RNA recovery.** Poor recovery of the Carrier RNA (0.5 ng/µL) could indicate a problem with the nucleic acid isolation process. See "Well-to-well variation in RNA/DNA yield" below for suggestions that may help with nucleic acid recovery. If these suggestions do not improve Carrier RNA recovery, the procedure may require further optimization for use with different sample types; contact Life Technologies' Technical Services Department for more information on how to optimize the kit for use with various sample types.

Well-to-well variation in RNA/DNA 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/DNA recovery may differ between different matrices. 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.

- Make sure the Bead Mix is fully resuspended before adding it to the Processing Plate at the start of the procedure (step 1 on page 10).
- Make sure that the RNA Binding Beads are fully resuspended in Elution Buffer to efficiently elute nucleic acids from the beads in step 8a on page 11. Fully resuspended beads will produce a homogenous 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. It may also be necessary to pipet the samples up and down to break up the beads.
- Avoid overdrying the RNA Binding Beads before eluting the RNA/DNA (step 7 on page 11) 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 (step 8 on page 11) 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/DNA. Avoid aspirating the RNA Binding Beads when removing supernatant from the captured beads.

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 when aspirating supernatant.

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 "RNA Binding Beads were unintentionally lost" on page 14 for suggestions for avoiding 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.

Supplemental Information

MagMAX™ Express viral RNA isolation protocol overview

The MagMAX™-96 Viral RNA Isolation procedure can be adapted for use with Life Technologies MagMAX™ Express-96 (for 1–96 samples per run) Standard and Deep Well Magnetic Particle Processors and the MagMAX™ Express (for 1–24 samples per run) Magnetic Particle Processor. Downloadable proctocols for these instruments are available on a Life Technologies automation resource page on the web:

www.lifetechnologies.com/magmaxexpress/scripts

MagMAX™ Express-96 purification using a Deep Well Magnetic Head

General guidelines for purification using the MagMAX™ Express-96 instrument equipped with a Deep Well Magnetic Head:

- Prepare the plates in the order outlined below.
- **Use a Deep Well plate for the sample plate to prevent sample cross-contamination.**
- It is critical to prepare the sample plate **last** to minimize the time that sample, Bead Mix, and Lysis/Binding Solution are unmixed.
- Immediately load the sample plate onto the MagMAX™ Express-96 instrument for purification to ensure best results.

Stepwise Procedure:

1. Select the "AM1836_DW_50v3" protocol on the MagMAX™ Express-96 instrument.
2. Load 150 µl/well Wash Solution 1 into two standard well plates.
3. Load 150 µl/well Wash Solution 2 into two standard well plates.
4. Load 90 µl/well of Elution Buffer into one standard well plate.
5. Load reagents/sample into the deep well sample plate with the following volumes and order:
   a. 20 µL/well Bead Mix
   b. 50 µL/well sample
   c. 130 µL/well Lysis/Binding Solution
6. Load all plates onto MagMAX™ Express-96 in the positions directed in the table below.
7. Start protocol.

<table>
<tr><th colspan="5">MagMAX™ Express-96 (MME-96) Instrument Setup</th></tr>
<tr><td colspan="3">MME-96 Protocol Name</td><td colspan="2">AM1836_DW_50v3</td></tr>
<tr><td colspan="3">Machine Configuration / Cat. no.</td><td colspan="2">MME-96 Deep Well Magnetic Head / 4388435</td></tr>
<tr><td colspan="3">Tip Comb / Cat. no.</td><td colspan="2">MME-96 Deep Well Tip Comb / 4388487</td></tr>
<tr><td colspan="3">Sample Volume</td><td colspan="2">50 µL</td></tr>
<tr><td colspan="2">Plate Position</td><td colspan="2">Reagent Addition Order and Volume/well</td><td>Plate to Use (Plate / Cat. no.)</td></tr>
<tr><td rowspan="3">1</td><td rowspan="3">Sample Plate</td><td>Bead Mix</td><td>20 µL</td><td rowspan="3">MME-96 Deep Well Plate / 4388476</td></tr>
<tr><td>Sample</td><td>50 µL</td></tr>
<tr><td>Lysis/Binding Soln.</td><td>130 µL</td></tr>
<tr><td>2</td><td>1st Wash 1</td><td>Wash Solution 1</td><td>150 µL</td><td>MME-96 Standard Plate / 4388475</td></tr>
<tr><td>3</td><td>2nd Wash 1</td><td>Wash Solution 1</td><td>150 µL</td><td>MME-96 Standard Plate / 4388475</td></tr>
<tr><td>4</td><td>1st Wash 2</td><td>Wash Solution 2</td><td>150 µL</td><td>MME-96 Standard Plate / 4388475</td></tr>
<tr><td>5</td><td>2nd Wash 2</td><td>Wash Solution 2</td><td>150 µL</td><td>MME-96 Standard Plate / 4388475</td></tr>
<tr><td>6</td><td>Elution</td><td>Elution Buffer</td><td>90 µL</td><td>MME-96 Standard Plate / 4388475</td></tr>
<tr><td>7</td><td>Tip Comb Plate</td><td colspan="2">Deep Well Tip Comb in Plate</td><td>MME-96 Standard Plate / 4388475</td></tr>
</table>

MagMAX™ Express (24-well) purification

Procedure for purification using MagMAX™ Express (24-well) Magnetic Particle Processor:

1. Select the "AM1836v2" protocol.
2. Insert tip combs into instrument head.
3. Load 150 µL/well Wash Solution 1 into Rows B and C of MagMAX™ Express plate. (See table below.)
4. Load 150 µL/well Wash Solution 2 into Rows D and E of MagMAX™ Express plate.
5. Load 90 µL/well of Elution Buffer to Row F of MagMAX™ Express plate.
6. Load in the following order into Row A:
   a. 20 µL/well NA binding bead mix
   b. 50 µL/well sample
   c. 130 µL/well Lysis/Binding Solution
7. Load sample plate onto MagMAX™ Express.
8. Start protocol.

MagMAX™ Express (MME) Instrument Setup			
MME Protocol Name			AM1836v2
Plate / Cat. no.			MME Plate / 4388474
Tip Comb / Cat. no.			MME Tip Comb / 4388452
Sample Volume			50 µL
Row Position		Reagent Addition Order and Volume/well	
A	Sample wells	Bead Mix	20 µL
		Sample	50 µL
		Lysis/Binding Soln.	130 µL
B	1st Wash 1	Wash Solution 1	150 µL
C	2nd Wash 1	Wash Solution 1	150 µL
D	1st Wash 2	Wash Solution 2	150 µL
E	2nd Wash 2	Wash Solution 2	150 µL
F	Elution	Elution Buffer	90 µL

Related products available from Life Technologies

Product	Description
RNase*Zap*® Solution **Cat. nos. 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. no. AM10050**	The 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. no. AM10027**	The 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. no. AM10050).
MagMAX™-96 Total RNA Isolation Kit **Cat. no. 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.
MagMAX™ Pathogen DNA/RNA Kit **Cat. no. 4462359**	The MagMAX™ Pathogen RNA/DNA Kit enables purification of RNA and DNA from viruses and easy-to-lyse bacteria and parasites, using magnetic particle technology in a 96-well format. PCR inhibitors are effectively removed in the process, making the resulting nucleic acid ideal for real-time PCR and RT-PCR applications.
VetMAX™ Plus One-Step RT-PCR Kit **Cat. no. 4415328**	The VetMAX™-Plus One-Step RT-PCR Kit is designed for robust amplification of animal pathogen RNA targets using a rapid, single-tube, real-time, reverse transcription PCR (RT-PCR) strategy. The kit includes Xeno™ RNA Control, which serves as an internal positive control for RNA purification and RT-PCR amplification. RNA is reverse-transcribed into cDNA, and then your target is amplified using AmpliTaq Gold® DNA Polymerase on a real-time PCR system.
VetMAX™ Plus Multiplex One-Step RT-PCR Kit **Cat. no. 4415330**	The VetMAX™-Plus Multiplex One-Step RT-PCR Kit is designed for multiplex amplification of animal pathogen RNA targets using a rapid, single-tube, real-time, reverse transcription PCR (RT-PCR) strategy. The kit includes Xeno™ RNA Control, which serves as an internal positive control for RNA purification and RT-PCR amplification. RNA is reverse-transcribed into cDNA, and then your target is amplified using AmpliTaq Gold® DNA Polymerase on a real-time PCR system.

Quality control

Functional testing

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

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

RNase activity: A sample is incubated with labeled RNA and analyzed by PAGE.

Nonspecific endonuclease activity: A sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity: A sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

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.

B Safety

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, and 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.

Specific chemical handling

CAS	Chemical	Phrase
593-84-0	Guanidine Isothiocyanate	Contact with acids or bleach liberates toxic gases. DO NOT ADD acids or bleach to any liquid wastes containing this product.

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. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
For support visit **lifetechnologies.com/support** or email **techsupport@lifetech.com**

lifetechnologies.com

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