MagMAX™ Total Nucleic Acid Isolation Kit

(Part Number AM1840)

Protocol

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I. Introduction

A. Product Description

The MagMAX™ Total Nucleic Acid Isolation Kit is designed for rapid high throughput purification of DNA and RNA for use in PCR and RT-PCR applications. A diverse range of sample types can be used with the kit:

- Biological samples
  - Feces and whole blood
  - Biofluids such as milk, urine, nasal fluids, swab samples, semen, sputum, and spent culture medium
  - Bacterial cultures and mammalian cell cultures
- Environmental samples
  - River and pond water
  - Agricultural samples such as manure pits and cow alleyways

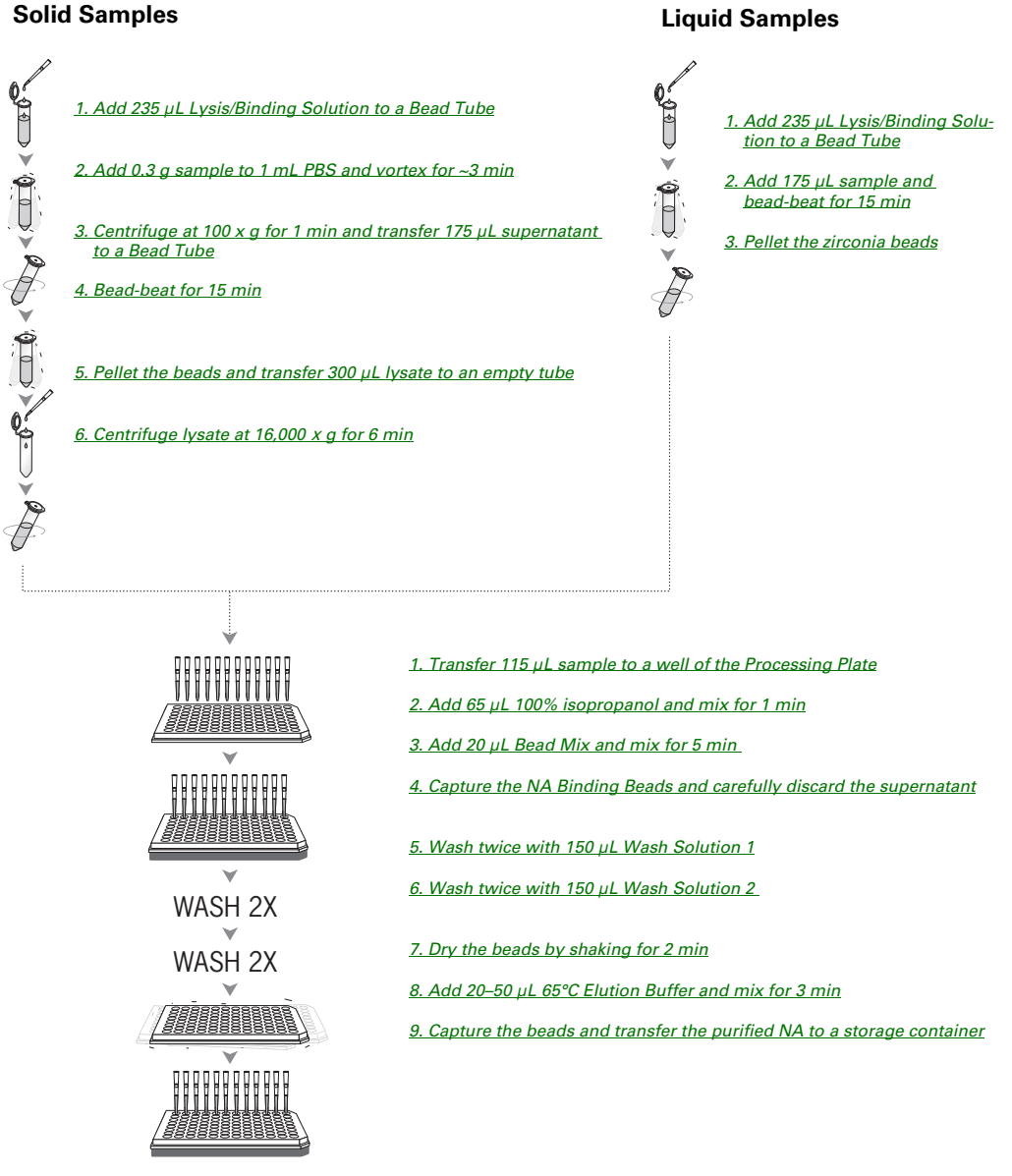
The procedure combines an effective method for disrupting samples that are difficult to lyse, such as *Mycobacterium*, with the MagMAX magnetic bead-based nucleic acid (NA) isolation and purification technology. The microspherical paramagnetic beads used in the kit have a large available binding surface and can be fully dispersed in solution, allowing thorough NA binding, washing, and elution. The procedure, therefore, delivers very consistent yields of high quality NA with >50% recovery and little sample-to-sample variation (recovery may vary depending on sample type). The entire MagMAX Total Nucleic Acid Isolation procedure requires approximately 1 hr.

B. Procedure Overview

The MagMAX Total Nucleic Acid Isolation Kit employs mechanical disruption of samples with zirconia beads in a guanidinium thiocyanate-based solution that rapidly releases NA while simultaneously inactivating nucleases in the sample matrix (Chirgwin, et al., 1979; Chomczynski and Sacchi, 1987) (Figure 1). Samples are then diluted with isopropanol, and paramagnetic beads with an NA binding surface are added to the sample. The beads/NA are immobilized on magnets and washed to remove proteins and other contaminants. A second wash solution is used to remove residual binding solution, and finally NA is eluted in a small volume of low-salt buffer.

The MagMAX Total Nucleic Acid Isolation procedure is designed for processing samples in 96-well plates, thus 96 samples can be processed at once; however, the kit can also be used to efficiently isolate RNA and DNA from fewer than 96 samples.

Figure 1. MagMAX Total Nucleic Acid Isolation Kit Procedure Overview



C. Reagents Provided with the Kit and Storage Conditions

The MagMAX Total Nucleic Acid Isolation Kit contains reagents to isolate RNA from 96 samples.

Amount	Component	Storage
360 µL	Carrier NA (1 mg/mL)	–20°C
1.1 mL	Lysis/Binding Enhancer	–20°C
1.1 mL	NA Binding Beads	4°C
40 mL	Wash Solution 2 Concentrate (Add 32 mL of 100% ethanol before use)	4°C or room temp
9 mL	Elution Buffer	4°C or room temp
28 mL	Lysis/Binding Solution Concentrate (See section II.A before use)	room temp
36 mL	Wash Solution 1 Concentrate (Add 12 mL 100% isopropanol before use)	room temp
2	Processing Plate and Lid	room temp
100	Bead tube	room temp
2 x 200 ml	1X PBS Buffer pH 7.4	any temp*

* Component may be stored at –20°C, 4°C, or room temp.

D. Required Materials Not Provided with the Kit

Lab equipment and supplies

- General laboratory equipment including microcentrifuge, pipettors, spectrophotometer, and RNase-free tips
- Cell disruption equipment for bead beating: the following instruments have been used successfully with this kit at Ambion:
  - Ambion® Vortex Adapter, P/N AM10024 with the Vortex-Genie® 2 (Scientific Industries) vortex mixer (the adapter is recommended only for this vortex mixer)
  - Disruptor Genie® Cell Disruptor/Homogenizer (Scientific Industries) with the TurboMix Attachment for 2 mL tubes
  - FastPrep®-24 Instrument (MP Biomedicals)
  - Precellys 24 (Bertin Technologies)
- Magnetic stand for 96-well plates: We recommend the Ambion 96-well Magnetic-Ring Stand (P/N AM10050) for its high strength magnets and quality design. Alternatively, most other magnetic stands for 96-well plates may be used.
- Multichannel pipettor
- (Optional but recommended) Repeating pipettor capable of dispensing the specified volume multiple times
- Orbital shaker for 96-well plates such as the Barnstead/Lab-Line Titer Plate Shaker (VWR #57019-600 or Fisher #14-271-9)

- If you process fewer than 96 samples at a time, you will need additional polystyrene U-bottom 96-well plates and lids.

Reagents

- 100% ethanol, ACS reagent grade or equivalent
- 100% isopropanol, ACS reagent grade or equivalent

Reagents/equipment for solid samples such as feces

To work with solid or particulate-rich samples such as feces, the following additional reagents and equipment are needed/recommended:

- (Fecal samples) Appropriate biohazard safety precautions may indicate that samples should be weighed and transferred in a hood. We recommend the Ohaus model HH 320 X 0.1 g balance for its compact footprint and low cost (VWR #WLS2655-32).
- 2 mL microcentrifuge tubes; we recommend USA Scientific Cat #1620-2700 because their wide opening accommodates a disposable spatula, facilitating sample transfer.
- Disposable spatula (for example VWR Cat #80081-190)

E. Related Products Available from Applied Biosystems

96-well Magnetic-Ring Stand P/N 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, practice may be required for efficient manual removal of supernatants.
Magnetic Stand-96 P/N 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 (P/N AM10050).
Electrophoresis Reagents See web or print catalog for P/Ns	Ambion offers gel loading solutions, agaroses, acrylamide solutions, powdered gel buffer mixes, nuclease-free water, and RNA and DNA molecular weight markers for electrophoresis. Please see our catalog or our website (www.ambion.com) for a complete listing as this product line is always growing.
RNase*Zap*® Solution P/N 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.

RNA*later*® Solution P/N AM7020, AM7021	RNA*later* Tissue Collection: RNA Stabilization Solution is an aqueous sample collection solution that stabilizes and protects cellular RNA in intact, unfrozen tissue and cell samples. RNA*later* Solution eliminates the need to immediately process samples or to freeze samples in liquid nitrogen. Samples can be submerged in RNA*later* Solution for storage at RT, 4°C, or –20°C without jeopardizing the quality or quantity of RNA that can be obtained.
MICROB*Enrich*™ Kit P/N AM1901	The MICROB*Enrich* Kit employs a novel technology to remove over 90% of mammalian RNA from complex mixtures of host-bacterial RNA samples. If desired, the enriched bacterial RNA obtained can be enriched for bacterial mRNA using the MICROB*Express* Kit
MICROB*Express*™ Kit P/N AM1905	The MICROB*Express* Kit employs a novel technology to remove >95% of the 16S and 23S rRNA from total RNA of a broad spectrum of gram-positive and gram-negative bacteria.

II. MagMAX™ Total Nucleic Acid Isolation Procedure

A. Reagent and Equipment Preparation

Before using the kit the first time, prepare wash solutions

- Add 12 mL 100% isopropanol to the bottle labeled Wash Solution 1 Concentrate and mix thoroughly. The resulting mixture is called Wash Solution 1 in these instructions.
- Add 32 mL 100% ethanol to the bottle labeled as Wash Solution 2 Concentrate and mix thoroughly. The resulting mixture is called Wash Solution 2 in these instructions.
- Mark the labels to indicate that the wash solutions are complete.
- Store the wash solutions at room temperature.

Determine maximum shaker speed for the procedure

Using ~210 µL of water per well of a 96-well plate, determine the maximum shaking speed that can be used with your orbital shaker without spilling sample. Use this speed for all shaking steps in the procedure.

Add Carrier NA to the Lysis/Binding Solution

IMPORTANT

Carrier NA is not known to affect RT-PCR, even when oligo(dT) is used as primer for the RT reaction. If you are isolating RNA with the intention of amplifying it for microarray analysis, however, Carrier NA could interfere with RNA amplification using oligo(dT) primers. Carrier NA can be left out of the Lysis/Binding Solution for all sample types, except cell-free or nearly cell-free samples; replace it with Nuclease-free Water. Carrier NA is required for quantitative NA recovery from cell-free or nearly cell-free samples, so this kit is not recommended for NA isolation from these sample types for use in microarray analysis.

IMPORTANT

Shipment on dry ice may cause the Carrier NA to become gelatinous and difficult to pipet. If you experience problems when attempting to pipet the Carrier NA, 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.

a. Prepare the amount of Lysis/Binding Solution needed for one month as shown in Table 1. If you prepare less than the entire bottle, include ~10% overage to cover pipetting loss. Once the Carrier NA is added, the solution is stable for one month.

Table 1. Lysis/Binding Solution Preparation

	Per Rxn	Entire Bottle
Lysis/Binding Solution Concentrate	232 µL	28 mL
Carrier NA (1 µg/sample)	3 µL	360 µL

b. Mix by inverting the tube several times.

- If you prepare the entire bottle, mark the label to indicate this.
- Store the prepared Lysis/Binding Solution at room temperature. Storage at lower temperatures may cause the Carrier NA to precipitate. If the solution is inadvertently stored at 4°C, warm it at 37°C and shake to dissolve any precipitates before use.

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 it on the day it will be used.

a. Prepare ***Bead Mix*** by as shown in Table 2 for the number of isolation reactions to be performed. Add ~10% overage to cover pipetting loss. Mix thoroughly by gently vortexing.

Table 2. Bead Mix preparation

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

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

b. Place the prepared Bead Mix on ice until it is needed in step II.C.3.

B. Sample Disruption

The sample disruption procedure is slightly different for solid samples such as feces, and liquid samples such as biofluids and bacterial cultures. Separate instructions are provided in sections B.I and B.II.

RNase decontamination

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 to perform this procedure and change them frequently. They will protect you from the reagents, and they will protect the RNA from nucleases that are present on skin.
- Use RNase-free tips to handle the wash solutions and the Elution Solution, and avoid putting used tips into the kit reagents.

B.I. Disruption of Solid Samples

Follow this sample disruption procedure for solid samples such as feces and cow alleyway samples.

1. Add 235 µL Lysis/Binding Solution to a Bead Tube

Dispense 235 µL Lysis/Binding Solution (prepared as described on page 6) into a Bead Tube for each sample. Set the tube(s) aside for use in step 4 below.

2. Add 0.3 g sample to 1 mL PBS and vortex for ~3 min

a. Mix samples thoroughly to create a homogenous sample. This is critical for detection of pathogens such as MAP, because the pathogens are typically concentrated in fecal material that was adjacent to the intestinal wall.

b. For each sample, place 1 mL of PBS in a 2 mL microcentrifuge tube.

c. Add 0.3 g (±0.1 g) sample to the PBS.

d. Mix by vortexing at moderate to high speed for 3 min to fully suspend the sample.

The volume of PBS provided is sufficient for relatively large fecal samples. To prepare larger fecal samples, scale up proportionally.

3. Centrifuge at 100 x g for 1 min and transfer 175 µL supernatant to a Bead Tube

a. Centrifuge samples at very low speed, 100 x g, for 1 min.

b. Transfer 175 µL of the supernatant to each Bead Tube containing Lysis/Binding Solution.
For isolation of nucleic acids from relatively large organisms such as *Mycobacterium*, it is important to transfer the supernatant before the cells settle to the bottom of the tube.

4. Bead-beat for 15 min

Bead-beat the samples for 15 min on a vortex mixer.

This step lyses cells to release nucleic acids by physical disruption with the zirconia beads. Adequate agitation is particularly important for cells that are difficult to lyse, such as *Mycobacterium*.

Following are recommendations for thorough sample disruption using the equipment tested at Applied Biosystems.

Table 3. Sample Disruption Recommendations

Vortex adapter & mixer	Vortex mixer speed and time	Comments
Ambion Vortex Adapter and Vortex-Genie® 2	Speed: maximum, Time: 15 min	Position the sample tubes horizontally on the vortex adapter with the tube caps towards the center.
Disruptor Genie®	Speed: maximum, Time: 15 min	Use the TurboMix attachment for 2 mL tubes.
FastPrep®-24 Instrument	Speed: 6.5 m/s, Time: 2 x 1 min	Two cycles of 1 min each. Allow at least 2 min rest between cycles.
Precellys 24	Speed: 6800 rpm, Time: 2 x 1.5 min	Two cycles of 1.5 min each. Allow 5 min rest between cycles.

5. Pellet the beads and transfer 300 µL lysate to an empty tube

a. Pellet the zirconia beads by centrifuging at 16,000 x g for 3 min.

b. Transfer 300 µL of lysate to a new, empty 1.5 mL microcentrifuge tube, being careful to avoid transferring the zirconia beads.

6. Centrifuge lysate at 16,000 x g for 6 min

a. Clarify the lysate by centrifuging at 16,000 x g for 6 min.

b. Proceed to section *C. Nucleic Acid Purification* starting on page 9.

B.II. Disruption of Liquid Samples

Follow this sample disruption procedure for liquid samples such as whole blood, milk, bacterial and cell cultures, and other biofluid samples.

1. Add 235 µL Lysis/Binding Solution to a Bead Tube

Dispense 235 µL Lysis/Binding Solution (prepared as described on page 6) into a Bead Tube for each sample.

2. Add 175 µL sample and bead-beat for 15 min

a. Add 175 µL of sample to each Bead Tube containing Lysis/Binding Solution.

b. Bead-beat the samples for 15 min on a vortex mixer.
This step lyses cells to release nucleic acids by physical disruption with the zirconia beads. Adequate agitation is particularly important for cells that are difficult to lyse, such as *Mycobacterium*. Table 3 on page 8 lists recommendations for thorough sample disruption using the vortex adapters tested at Ambion.

3. Pellet the zirconia beads

a. Pellet the zirconia beads by centrifuging at 16,000 x g for 3 min.

b. Proceed to section *C. Nucleic Acid Purification* below.

C. Nucleic Acid Purification

Before starting the NA purification:
Place the Elution Buffer at 65°C to preheat it for the final NA elution in step 8.

1. Transfer 115 µL sample to a well of the Processing Plate

Transfer 115 µL of sample supernatant to a well of the Processing Plate.

IMPORTANT

When working with solid samples, aspirate the sample supernatant carefully to avoid carryover of the pelletted material to the Processing Plate.

NOTE

If the NA will be used to detect DNA targets by RT-PCR, unused sample supernatant can be stored at room temperature for up to 5 days. Do not store unused sample lysate below room temperature as this may reduce NA yield due to precipitation.

2. Add 65 µL 100% isopropanol and mix for 1 min

a. Add 65 µL 100% isopropanol (ACS reagent grade or equivalent) to each sample in the Processing Plate.

b. Shake the plate for 1 min on an orbital multiwell-plate shaker at the maximum speed identified in step *A. Determine maximum shaker speed for the procedure* on page 6.

3. Add 20 µL Bead Mix and mix for 5 min

a. Vortex the prepared Bead Mix at moderate speed to create a uniform suspension before pipetting (prepared as described in step A. *Prepare Bead Mix* on page 7).

b. Remove the plate from the shaker and add 20 µL Bead Mix to each sample.

c. Shake the plate for 5 min to bind NA to the NA Binding Beads.

4. Capture the NA Binding Beads and carefully discard the supernatant

a. Move the Processing Plate to a magnetic stand to capture the NA Binding Beads. Leave the plate on the magnetic stand for 3–5 min. When capture is complete, the NA 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, and remove the Processing Plate from the magnetic stand.

IMPORTANT

To obtain pure NA, it is important to completely remove the supernatant at this step; use the Ambion 96-well Magnetic-Ring Stand (P/N AM10050) for the best consistency.

5. Wash twice with 150 µL Wash Solution 1

a. Remove the plate from the shaker and add 150 µL Wash Solution 1 (isopropanol added) to each sample and shake the plate for 1 min.

b. Capture the NA 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 and remove the Processing Plate from the magnetic stand. It is critical to move the Processing Plate off the magnetic stand before the subsequent wash.

d. Repeat steps a–c to wash a second time with 150 µL Wash Solution 1.

6. Wash twice with 150 µL Wash Solution 2

a. Remove the plate from the shaker and add 150 µL Wash Solution 2 (ethanol added) to each sample and shake for 1 min.

b. Capture the NA 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 a–c to wash a second time with 150 µL Wash Solution 2.

IMPORTANT

It is important to completely remove Wash Solution 2 from the samples, because otherwise it could inhibit downstream applications such as RT-PCR.

7. Dry the beads by shaking for 2 min

a. Move the Processing Plate to the shaker and shake for 2 min 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 >5 min, as this could overdry the beads.

8. Add 20–50 µL 65°C Elution Buffer and mix for 3 min

a. Add 20–50 µL 65°C Elution Buffer to each sample, and shake vigorously for 3 min.

NOTE

The elution volume is somewhat flexible; NA can be eluted as little as 20 µL, or in >50 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. Inspect the samples to make sure that the magnetic beads are uniformly resuspended in the Elution Buffer. If aggregates of beads are visible, pipette up and down ~10 times to thoroughly resuspend the beads, then shake for 1 more minute. Complete resuspension of beads is critical for consistent and efficient NA recovery.

9. Capture the beads and transfer the purified NA to a storage container

a. Capture the NA Binding Beads as in the previous steps. ***The purified NA will be in the supernatant***.

b. Transfer the supernatant, which contains the NA, to a nuclease-free container appropriate for your application, and store the purified NA at –20°C.

D. Analyzing NA Recovery

This kit is designed for NA purification for use in PCR and RT-PCR with real-time detection of amplification products. By including positive controls, NA obtained with the kit can be used directly in PCR/RT-PCR without first analyzing RNA yield or recovery.

If your application requires analysis of NA samples, the appropriate technique will depend somewhat on the amount of material that you expect to be recovered. For example, samples containing many cells, such as bacterial cultures or whole blood, can be analyzed by spectrophotometry to determine yield, and by an Agilent bioanalyzer to evaluate quality. On the contrary, cell-free or nearly cell-free samples contain very little NA, thus the majority of NA recovered will be the Carrier NA that was added to the Lysis/Binding Solution. For these types of samples, we recommend determining the quantity of Carrier NA recovered because that will provide a good indication of overall nucleic acid recovery.

NA quantitation

The concentration of a NA solution can be determined by measuring its UV absorbance at 260 nm (A_{260}). We recommend the NanoDrop 1000A Spectrophotometer for its convenience. No dilutions or cuvettes are needed; just measure 1.5 µL of the NA sample directly.

Low cell content samples

For samples with very low cell content, such as plasma and serum, the Carrier NA will contribute the majority of the A_{260} measurement. 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

Fecal samples

Humic acid is a major component of fecal samples, and it absorbs UV light at 260 nm. NA isolated from these sample types will typically contain trace amounts of humic acid which will make NA quantitation by UV absorbance unreliable.

Other sample types

For other sample types, a combination of RNA and DNA concentration will be measured using the A_{260}. In order to determine the concentration of RNA or DNA exclusively, we recommend using a fluorometric agent such as Ribogreen or Picogreen.

Expected recovery of the Carrier NA

With most sample types, ≥50% of the input NA should be recovered, but recovery may be lower with some sample matrices. Each sample is lysed in Lysis/Binding Solution containing 3 µg Carrier NA per sample, but only a portion of the lysate is carried through the entire procedure. Using the recommended volumes of sample at each step, each sample will contain ~840 ng of Carrier NA, therefore >420 ng of Carrier NA should be recovered.

III. Troubleshooting

A. Poor NA Detection by PCR or RT-PCR

If target NA cannot be detected by PCR or RT-PCR, or amplification reactions yield a higher than expected C_T, it may indicate problems with the RT-PCR reagents or equipment used, nucleic acid recovery, or reaction inhibitors present in the NA. In the following sections troubleshooting ideas for the latter two issues are described.

B. Troubleshooting Inhibitors of RT and/or PCR

With most samples, the MagMAX Total Nucleic Acid Isolation Kit protocol yields very pure nucleic acid; however, with samples that contain excessively high amounts of reaction inhibitors, enough may be carried over to inhibit PCR or RT-PCR.

A control nucleic acid can be added to the Lysis/Binding Solution in step II.A on page 6 to detect inhibitors. If amplification of the added control nucleic acid fails, reaction inhibitors are most likely present in the NA sample.

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

The effect of inhibitors can be minimized or eliminated by reducing the amount of NA used in PCR or RT-PCR. Following are suggestions listed in the order of recommended use.

1. Use a smaller volume, for example 1–2 µL of NA, in the reverse transcription reaction or PCR.
2. Dilute the purified NA 10-fold and repeat the RT-PCR or PCR.
3. Dilute the sample lysate 4-fold and use the diluted lysate for NA isolation.
   - Prepare a *lysate dilution solution* containing 175 µL PBS and 235 µL Lysis/Binding Solution (containing Carrier NA).
   - Mix 28 µL lysate (after completing the clarification, step II.B.6 on page 8 or the zirconia bead centrifugation, step II.B.3 on page 9) with 87 µL *lysate dilution solution*.
   - Continue with the NA isolation procedure.
4. Dilute the sample 2-fold in PBS before starting the NA isolation procedure.
   For example, with solid samples, complete the low speed centrifugation in step II.B.3 on page 8, transfer the supernatant to a fresh tube and dilute with an equal volume of PBS. Vortex for 3 min, then complete the NA isolation procedure, starting at step II.B.3 on page 8.

C. Troubleshooting NA Recovery

Poor cell disruption

To obtain the maximum NA yield, samples must be agitated with zirconia beads with sufficient force and time to lyse the cells. Otherwise NA yield will be compromised due to insufficient cell lysis. This may be seen with hard to lyse samples such as *Mycobacterium*.

Different vortex adapters will provide different amounts of force in the tube. The adapters and recommended times and speeds tested at Applied Biosystems are provided in Table 3 on page 8. Other equipment may or may not provide the force needed to lyse difficult samples. If cell disruption is a problem, the incubation time of the bead-beating step (step II.B.4 on page 8 or II.B.2 on page 9) can be increased from 15 min to 30 min.

Cell-free or nearly cell-free samples

NA yield from cell-free or nearly cell-free samples such as urine and cell culture medium will be very low. With these sample types, NA yield can only be evaluated by PCR/RT-PCR results and recovery of the Carrier NA that is added to the Lysis/Binding Solution.

Good recovery of Carrier NA, but NA of interest is not detected

If the Carrier NA was recovered at expected levels (≥420 ng/sample), but a pathogen NA cannot be detected in a known positive sample using a proven qRT-PCR or qPCR assay system, troubleshoot problems with sample lysis (see above), or with the RT-PCR or PCR reagents and/or equipment. Also, consider the recommendation in the previous section for diluting your sample to minimize the effects of inhibitors.

Lower-than-expected Carrier NA recovery

Poor recovery of the Carrier NA could indicate a problem with the nucleic acid isolation process. The following suggestions may help with nucleic acid recovery.

Well-to-well variation in NA yield

The efficiency of NA recovery may differ among sample types, but NA yield should be fairly uniform between wells of a 96-well plate with the same sample type. If large variations in NA yield are observed among samples of the same type, consider the following suggestions:

NA Binding Beads were not fully resuspended/dispersed

In general, the NA Binding Beads will disperse more easily when the temperature of the mixture is warmer than 20°C. Also, using the Ambion® 96-well Magnetic-Ring Stand (P/N AM10050) results in captured magnetic bead pellets that are easier to resuspend than magnetic beads captured using other devices.

a. Make sure the Bead Mix is fully resuspended before adding it to the Processing Plate at the start of the procedure (step II.C.3 on page 10).

b. Make sure that the NA Binding Beads are fully resuspended in Elution Buffer to efficiently elute nucleic acids from the beads in step II.C.8 on page 11. Fully resuspended beads will produce a homogenous brown solution. If the solution is clear, with brown clumps, the beads are not fully resuspended. Preheated Elution Buffer (60–65°C) will facilitate resuspension of the beads, and if that is not sufficient, shake the plate for an additional minute, or until the solution is homogenous before proceeding.

c. Avoid overdrying the NA Binding Beads in step II.C.7 because it may make the beads more difficult to resuspend in the final NA elution. If the beads are inadvertently overdried, increase the mixing time to 10 min during the elution step to allow the beads to rehydrate.

NA Binding Beads were unintentionally lost

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

To prevent aspiration of NA 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 NA Binding Beads when aspirating supernatant.

D. NA Binding Bead Carryover

If NA 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 PCR.

- To avoid bead carryover, see *NA Binding Beads were unintentionally lost* on page 15.
- To remove NA Binding Beads from NA samples, capture them on a magnetic stand for ~1 min. Then transfer the NA solution(s) to a fresh nuclease-free plate or tubes.

IV. Appendix

A. 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 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.

C. Quality Control

Functional Testing

Kit components are tested functionally by isolating the carrier NA and an Armored RNA using the procedure described in this protocol.

RNA recovery is assessed by absorbance measurements using a Nano-Drop Spectrophotometer and by qRT-PCR.

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

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

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

Exonuclease activity
Meets or exceeds specification when a sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

Protease testing

Meets or exceeds specification when a sample is incubated with protease substrate and analyzed by fluorescence.