applied biosystems

MagMAX™ Total Nucleic Acid Isolation Kit

USER GUIDE

Catalog Number AM1840

Publication Number MAN0017827

Revision B

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0017827 B (English)

Revision	Date	Description
B	6 May 2024	The regulatory statement was updated to match the product labels.
A.0	23 April 2018	• Initial release with new publication number format. • Supersedes publication number 1840M. • Updated to the current document template, with associated updates to trademarks, logos, licensing, and warranty.

The information in this guide is subject to change without notice.

Contents

1 Product information

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 Kit procedure requires approximately 1 hour.

Contents and storage

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

Contents	Amount	Storage
Processing Plate and Lid	2	15–30°C
Lysis/Binding Solution Concentrate	28 mL	15–30°C
Wash Solution 1 Concentrate[1]	36 mL	15–30°C
Wash Solution 2 Concentrate[1]	40 mL	4–30°C
Elution Buffer	9 mL	4–30°C
NA Binding Beads	1.1 mL	4°C
Bead tube	100	15–30°C
Carrier NA (1 mg/mL)	360 µL	–20°C
Lysis/Binding Enhancer	1.1 mL	–20°C
1X PBS Buffer pH 7.4	2 × 200 mL	–20°C to 30°C

[1] See "Before first use of the kit" on page 9 for preparation instructions.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Equipment	
Orbital shaker (96-well plates)	MLS
Magnetic stand, one of the following: • Magnetic-Ring Stand (96 well) • Magnetic Stand-96	• AM10050 • AM10027
Bead mill homogenizer for bead-beating, one of the following, or equivalent: • Fisherbrand™ Bead Mill 24 Homogenizer • Mixer Mill MM 400 (Verder 207450001) • Precellys™ 24 Homogenizer (Bertin) • FastPrep-24™ Instrument (MP Biomedical 116004500) • Mini-BeadBeater-96 (Glen Mills)	• Fisher Scientific™ 15-340-163 • Fisher Scientific™ 08418241 • Bertin EQ03119.200.RD000.0 • Fisher Scientific™ MP116004500 • Fisher Scientific™ NC0141170
Laboratory mixer, Vortex or equivalent	MLS

(continued)

Item	Source
Plates, tips and accessories	
96-well U-bottom plates and lids	thermofisher.com/plastics
RNAse-free pipette tips	thermofisher.com/pipettetips
Reagents	
100% ethanol, ACS grade or better	MLS
100% isopropanol, ACS grade or better	MLS
RNase*Zap*™ RNase Decontamination Solution	AM9780

Table 1 Additional materials for solid samples

Item	Source
2-mL tube	MLS
LevGo smartSpatula™ Disposable Polypropylene Spatula, Blue	Fisher Scientific™ 18-001-017

Overview of the procedure

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 (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, then finally NA is eluted in a small volume of low-salt buffer.

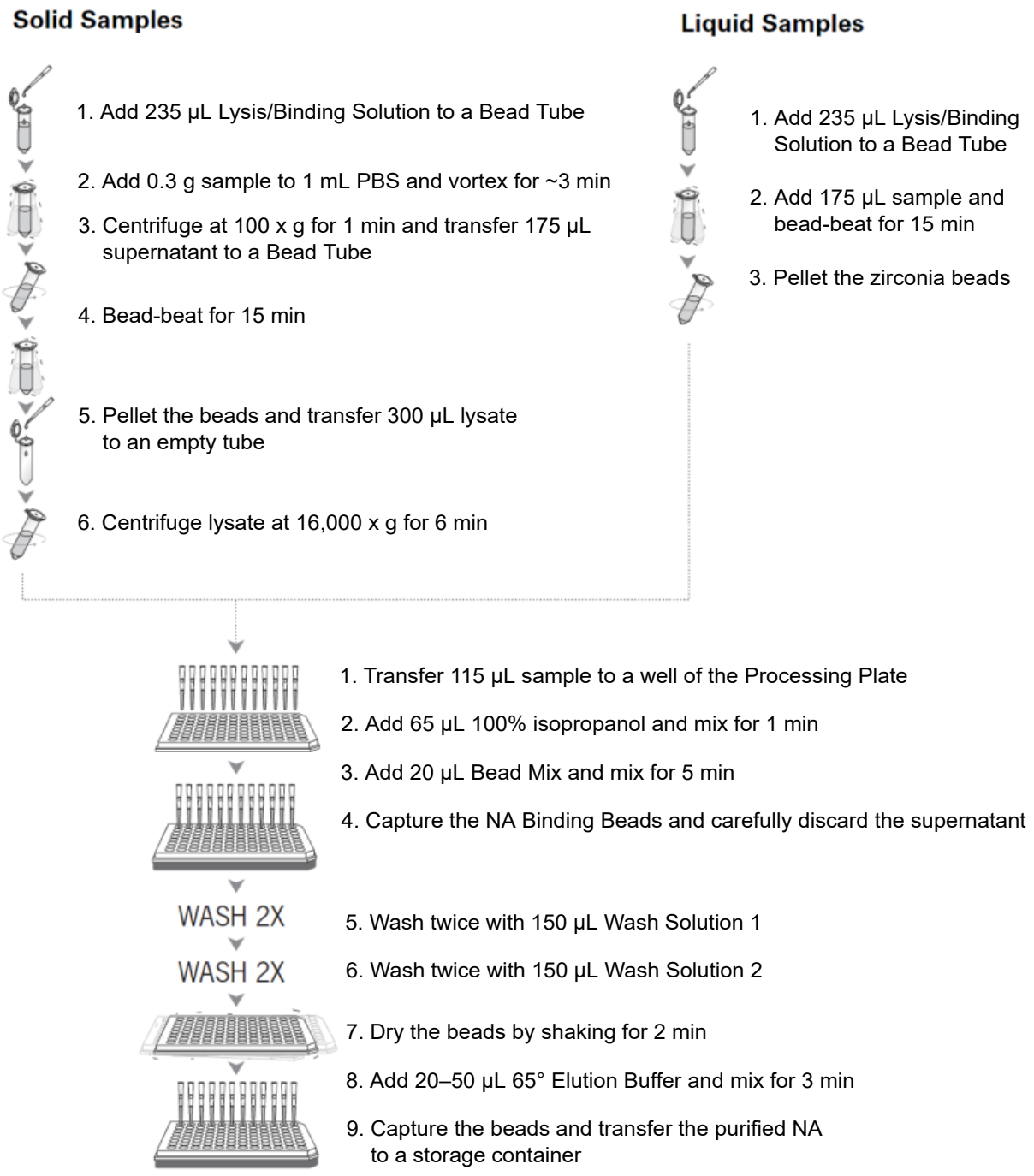

Figure 1 MagMAX™ Total Nucleic Acid Isolation Kit procedure overview

The MagMAX™ Total Nucleic Acid Isolation Kit 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.

2 Methods

Guidelines for nucleic acid isolation

- Before working with RNA, clean the lab bench and pipettors with an RNase decontamination solution (for example, RNase*Zap*™ RNase Decontamination Solution).
- Wear laboratory gloves for this procedure; 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.

Before you begin

Before first use of the kit

- 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 the maximum plate shaker setting

Using approximately 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 almost cell-free samples. Replace Carrier NA with Nuclease-free water. Carrier NA is required for quantitative NA recovery from cell-free or almost 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 can cause the Carrier NA to become gelatinous and difficult to pipette. If you experience problems when attempting to pipette the Carrier NA, we recommend heating it in a hybridization oven at 37°C for 10–15 minutes. A heat block can be used for this incubation, as long as the block accommodates the tube, and allows uniform heating of the solution to 37°C. After heating, vortex vigorously, then centrifuge briefly.

1. Prepare the amount of Lysis/Binding Solution that is needed for one month as shown in Table 2 on page 10. If you prepare less than the entire bottle, include 10% overage to cover pipetting loss. After the Carrier NA is added, the solution is stable for one month.

Table 2 Lysis/Binding solution preparation

Component	Volume per reaction	Volume per bottle
Lysis/Binding Solution Concentrate	232 µL	28 mL
Carrier NA (1 µg/sample)	3µL	360 µL

2. Mix by inverting the tube several times.
   - If you prepare the entire bottle, mark the label to indicate that.
   - Store the prepared Lysis/Binding Solution at room temperature. Storage at lower temperatures can 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 the 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 is used.

1. Prepare the Bead Mix by as shown in Table 3 on page 10 for the number of isolation reactions to be performed. Add 10% overage to cover pipetting loss. Mix thoroughly by gently vortexing.

Table 3 Bead Mix preparation

Component	Volume per reaction	Volume per plate (96 reactions + 10% overage)
NA Binding Beads[1]	10 µL	1.1 mL
Lysis/Binding Enhancer	10 µL	1.1 mL

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

2. Place the prepared Bead Mix on ice until it is needed in “Purify the nucleic acid” on page 13.

Disrupt the samples

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 "Disruption of solid samples" on page 11 and "Disruption of liquid samples" on page 12.

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 in "Add Carrier NA to the Lysis/Binding Solution" on page 10) into a Bead Tube for each sample. Set the tubes aside for use in step 4 on page 11.

2. Add 0.3-g sample to 1 mL PBS, then vortex for approximately 3 minutes.
   a. Mix samples thoroughly to create a homogeneous sample.

      IMPORTANT! This step is critical for detection of pathogens such as MAP, because the pathogens are typically concentrated in fecal material that was next 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. Vortex at moderate to high speed for 3-minutes to mix the sample.

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

3. Centrifuge at 100 × *g* for 1 minute, then transfer 175 µL of supernatant to a Bead Tube.
   a. Centrifuge samples at low speed, 100 × *g*, for 1 minute.

   b. Transfer 175 µL of the supernatant to each Bead Tube containing Lysis/Binding Solution.

      IMPORTANT! 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 the samples.

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

   Follow the recommended settings for maximum nucleic acid yield.

Option	Settings
Fisherbrand™ Bead Mill 24 Homogenizer	6 m/s; 3 minutes
Mixer Mill MM 400	30 Hz; 10 minutes
Precellys™ 24 Homogenizer	6,800 rpm; 2 × 90 seconds (Wait 5 minutes between cycles)
FastPrep-24™ Instrument	6.5 M/s; 2 × 60 seconds (Wait 5 minutes between cycles)
Mini-BeadBeater-96	5 minutes

Note: If an alternative instrument is used, follow the manufacturer's guidelines to determine the speed and time settings necessary to achieve sufficient cell lysis.

5. Collect the zirconia beads at the bottom of the tube, then transfer 300 µL of lysate to an empty tube.
   a. Centrifuge samples at 16,000 × *g* for 3 minutes to collect the zirconia beads at the bottom of the tube.
   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 the lysate at 16,000 × *g* for 6 minutes.
   a. Clarify the lysate by centrifuging at 16,000 × *g* for 6 minutes.
   b. Proceed to "Purify the nucleic acid" on page 13.

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 in "Add Carrier NA to the Lysis/Binding Solution" on page 10) into a Bead Tube for each sample.
2. Add 175 µL of sample and bead-beat the samples.
   a. Add 175 µL of sample to each Bead Tube containing Lysis/Binding Solution.
   b. Bead-beat the samples.

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

Follow the recommended settings for maximum nucleic acid yield.

Option	Settings
Fisherbrand™ Bead Mill 24 Homogenizer	6 m/s; 3 minutes
Mixer Mill MM 400	30 Hz; 10 minutes
Precellys™ 24 Homogenizer	6,800 rpm; 2 × 90 seconds (Wait 5 minutes between cycles)
FastPrep-24™ Instrument	6.5 M/s; 2 × 60 seconds (Wait 5 minutes between cycles)
Mini-BeadBeater-96	5 minutes

Note: If an alternative instrument is used, follow the manufacturer's guidelines to determine the speed and time settings necessary to achieve sufficient cell lysis.

3. Collect the zirconia beads at the bottom of the tube.
   a. Centrifuge samples at 16,000 × *g* for 3 minutes to collect the zirconia beads at the bottom of the tube.
   b. Proceed to "Purify the nucleic acid" on page 13.

Purify the nucleic acid

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

1. 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 pelleted material to the Processing Plate.

   Note: If the NA is 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 because this can reduce NA yield due to precipitation.

2. Add 65 µL of 100% isopropanol, then mix for 1 minute.
   a. Add 65 µL of 100% isopropanol (ACS reagent grade or equivalent) to each sample in the Processing Plate.
   b. Shake the plate for 1 minute on an orbital multiwell-plate shaker at the maximum speed that is identified in "Determine the maximum plate shaker setting" on page 9.
3. Add 20 µL of Bead Mix, then mix for 5 minutes.
   a. Vortex the prepared Bead Mix at moderate speed to create a uniform suspension before pipetting (prepared as described in "Prepare the Bead Mix" on page 10).
   b. Remove the plate from the shaker, then add 20 µL of Bead Mix to each sample.
   c. Shake the plate for 5 minutes 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 minutes. When the capture is complete, the NA Binding Beads 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, then remove the Processing Plate from the magnetic stand.

   IMPORTANT! To obtain pure NA, completely remove the supernatant at this step; use the Magnetic-Ring Stand (96 well) (Cat. No. AM10050) for the best consistency.

5. Wash twice with 150 µL Wash Solution 1.
   a. Remove the plate from the shaker, add 150 µL Wash Solution 1 (isopropanol added) to each sample, then shake the plate for 1 minute.

   b. Capture the NA Binding Beads on a magnetic stand for 1 minute, or until the mixture becomes clear, indicating that the capture is complete.

   c. Carefully aspirate and discard the supernatant without disturbing the beads, then 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 substep 5a to substep 5c 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, add 150 µL Wash Solution 2 (ethanol added) to each sample, then shake for 1 minute.

   b. Capture the NA Binding Beads as in the previous wash.

   c. Carefully aspirate and discard the supernatant without disturbing the beads, then 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 substep 6a to substep 6c to wash a second time with 150 µL Wash Solution 2.

   IMPORTANT! Completely remove Wash Solution 2 from the samples, otherwise it could inhibit downstream applications such as RT-PCR.

7. Dry the beads by shaking for 2 minutes.
   a. Move the Processing Plate to the shaker, then shake for 2 minutes to allow any remaining alcohol from the Wash Solution 2 to evaporate.

   b. Inspect the wells and if there is remaining solution, shake the plate for another 1–2 minutes to let it evaporate.

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

8. Add 20–50 µL of 65°C Elution Buffer, then mix for 3 minutes.
   a. Add 20–50 µL of 65°C Elution Buffer to each sample, then shake vigorously for 3 minutes.

   Note: The elution volume is flexible; NA can be eluted in as little as 20 µL, or in >50 µL to achieve the desired final nucleic acid concentration. The volume of Elution Buffer that is supplied with the kit is sufficient for 96 samples at 90 µL each.

   b. Inspect the samples to ensure that the magnetic beads are uniformly resuspended in the Elution Buffer. If aggregates of beads are visible, pipette up-and-down 10 times to resuspend the beads, then shake for 1 minute. Complete resuspension of beads is critical for consistent and efficient NA recovery.

9. Capture the beads, then transfer the purified NA to a storage container.
   a. Capture the NA Binding Beads as in the previous steps. **The purified NA is in the supernatant.**

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

Guidelines for analysis of 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 depends somewhat on the amount of material that you expect to be recovered.
  - 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.
  - Cell-free or almost cell-free samples contain little NA, therefore most NA recovered is 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 provides a good indication of overall nucleic acid recovery.

Quantify recovered NA

The concentration of a NA solution can be determined by measuring its UV absorbance at 260 nm (A_{260}). We recommend the NanoDrop™ 2000/2000c Spectrophotometer for its convenience. Absorbance readings using the NanoDrop™ are fast and easy because 1.5 µL can be measured without dilution, and no cuvettes are needed.

With most sample types, ≥50% of the input NA should be recovered, but recovery can 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 contains approximately 840 ng of Carrier NA, therefore > 420 ng of Carrier NA should be recovered.

Low cell content samples

For samples with low cell content, such as plasma and serum, the Carrier NA constitutes 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} × dilution factor × 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 typically contains trace amounts of humic acid, which makes NA quantification by UV absorbance unreliable.

Other sample types

For other sample types, a combination of RNA and DNA concentration will be measured using A_{260}. In order to determine the concentration of RNA or DNA exclusively, we recommend using a fluorometric agent such as Quant-iT™ RiboGreen™ RNA Reagent (Cat. No. R11491) or Quant-iT™ PicoGreen™ dsDNA Reagent (Cat. No. P7581).

A

Troubleshooting

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.

Inhibitors of RT-PCR or PCR

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

A control nucleic acid can be added to the Lysis/Binding Solution to detect inhibitors (see “Add Carrier NA to the Lysis/Binding Solution” step 1 on page 10). If amplification of the added control nucleic acid fails, reaction inhibitors are most likely present in the NA sample.

The effect of inhibitors can be minimized or eliminated by reducing the amount of NA used in PCR or RT-PCR. Following are suggestions that are 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 with 87 µL lysate dilution solution (after completing the clarification in “Disruption of solid samples” step 6 on page 12, or the zirconia bead centrifugation in “Disruption of liquid samples” step 3 on page 13).
    - 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 “Disruption of solid samples” step 3 on page 11, transfer the supernatant to a fresh tube, and dilute with an equal volume of PBS. Vortex for 3 minutes, then complete the NA isolation procedure.

Poor 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 mixers will provide different amounts of force in the tube. The recommended times and speeds tested are provided in "Disruption of solid samples" step 4 on page 11 and "Disruption of liquid samples" step 2 on page 12. Other equipment may or may not provide the force needed to lyse difficult samples. If cell disruption is a problem, increase the bead-beating speed or time in 25% increments (see step 4 on page 11 or step 2 on page 12).

Cell-free or almost cell-free samples

NA yield from cell-free or almost cell-free samples such as urine and cell culture medium will be 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 "Poor cell disruption" on page 18), 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 (see "Inhibitors of RT-PCR or PCR" on page 17).
- **Lower-than-expected carrier NA recovery.** Poor recovery of the Carrier NA could indicate a problem with the nucleic acid isolation process. See "Well-to-well variation in NA yield" on page 18 for suggestions that can 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 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

In general, the NA Binding Beads disperse more easily when the temperature of the mixture is warmer than 20°C. Also, using the Magnetic-Ring Stand (96 well) (Cat. No. AM10050) results in capturing magnetic bead pellets that are easier to resuspend than magnetic beads captured using other devices.

1. Ensure the Bead Mix is fully resuspended before adding it to the Processing Plate at the start of the procedure.
2. Ensure that the NA Binding Beads are fully resuspended in Elution Buffer to efficiently elute nucleic acids from the beads. Fully resuspended beads produce a homogeneous brown solution. If the solution is clear with brown clumps, the beads are not fully resuspended. Preheating the Elution Buffer to 60–65°C just before use helps resuspension of the beads. If that is not sufficient, shake the plate for an extra minute, or until the solution is homogeneous before proceeding.
3. Avoid overdrying the NA Binding Beads before eluting because it can make the beads more difficult to resuspend in the final NA elution. If the beads are inadvertently overdried, increase the mixing time to 10 minutes during the elution step to allow the beads to rehydrate.

NA binding beads were unintentionally lost

Because the principle of this procedure is to immobilize nucleic acids on the NA Binding Beads, any loss of beads during the procedure results 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 can 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, it appears light brown in color.

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.

NA binding bead carryover

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

- See “NA binding beads were unintentionally lost” on page 19 for suggestions to avoid bead carryover.
- To remove NA Binding Beads from NA samples, place the Processing Plate on magnetic stand to capture the beads for 1 minute. Then, transfer the NA solution to a fresh nuclease-free plate or tube.

B

Safety

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

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

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

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

Biological hazard safety

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

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

C Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.