USER GUIDE

ambion®
by life technologies™

MagMAX™-96 Total RNA Isolation Kit

Publication Part Number 1830M Rev. C
Revision Date November 2011

life technologies™

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

Information in this document is subject to change without notice.

LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

NOTICE TO PURCHASER: LIMITED USE LABEL LICENSE: Research Use Only

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

TRADEMARKS

The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners. Bioanalyzer and Agilent are trademarks of Agilent Technologies, Inc. LabChip is a trademark of Caliper Life Sciences, Inc.

Part Number 1830M Rev. C
November 2011

Contents

About This Guide

WARNING! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

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

Purpose

The *MagMAX™-96 Total RNA Isolation Kit User Guide* provides detailed procedures, reference information and troubleshooting for the kit.

User attention words

Five user attention words may appear in this document. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation or accurate chemistry kit use.

CAUTION! Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Except for IMPORTANTs, the safety alert words in user documentation appear with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instrument. See the "Safety" appendix for descriptions of the symbols.

MagMAX™-96 Total RNA Isolation Kit

Introduction

Product Description

The MagMAX™-96 Total RNA Isolation Kit is designed for rapid high throughput purification of total RNA in 96-well plates. The kit is optimized for use with either manual multichannel pipettors or with robotic liquid handlers. The microspherical magnetic beads used in this procedure have a large available binding surface and can be fully dispersed in solution, allowing thorough RNA binding, washing, and elution. The procedure, therefore, delivers high quality RNA with very consistent yield.

High yield of total RNA can be obtained with the MagMAX™-96 Total RNA Isolation Kit from the following sample types and quantities:

- 25 to 2 x 10^6 cultured mammalian cells—RNA yield is typically 5–20 pg RNA per cell, depending on the cell type and growth conditions.
- ≤ 5 mg animal tissue
- ≤ 5–10 mg plant tissue

The MagMAX™-96 Total RNA Isolation Kit can also be used for isolation of viral RNA from these sample types. For viral RNA isolation from biological fluids, such as serum, plasma, and swabs, we recommend using the MagMAX™-96 Total RNA Isolation Kit (Cat #AM1835).

Overview of the Procedure

The MagMAX™-96 Total RNA Isolation Kit employs a classic method for disrupting samples in a guanidinium thiocyanate-based solution that rapidly solubilizes cellular membranes and simultaneously inactivates nucleases (Chirgwin et al., 1979; Chomczynski and Sacchi 1987). Optimized protocols and disruption/lysis formulations are provided to achieve maximum RNA yield from cultured mammalian cells, animal tissues, and plant tissues. After homogenization, samples are mixed with magnetic beads that have a nucleic acid binding surface. The beads and bound nucleic acids are then magnetically captured and washed to remove cell debris, protein, and other contaminants. Next, the nucleic acid is treated with DNase and is purified from the reaction mixture. Finally, RNA is eluted in 50 µL of low salt buffer.

Figure 1 MagMAX™-96 Total RNA Isolation Kit Procedures

Sample Homogenization

Cultured mammalian cells

1. "Prepare Lysis/Binding Solution" on page 11
2. "Lyse up to 2 x 10^6 cells in 140 µL prepared Lysis/Binding Solution" on page 12
3. "Shake for 1 min on an orbital shaker at the maximum speed for larger volumes identified in step 2. on page 10. Cells will lyse during this shaking incubation." on page 12

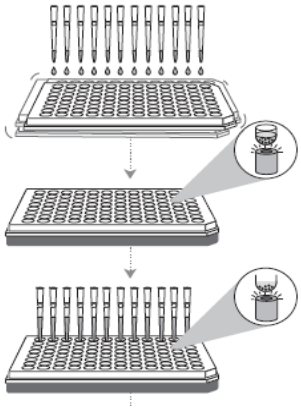
Animal tissue samples

1. "Prepare Lysis/Binding Solution" on page 12
2. "Homogenize up to 5 mg of tissue in 100 µL prepared Lysis/Binding Solution" on page 12
3. "Add 60 µL100% isopropanol and shake for 1 min" on page 13

Plant tissue samples

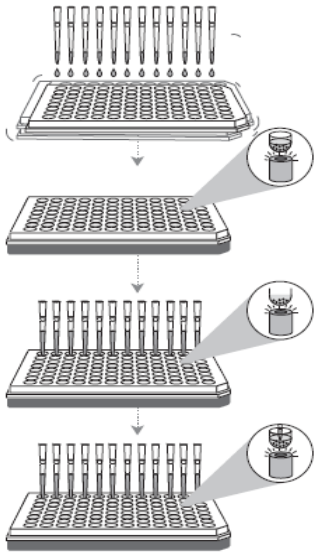
1. "Prepare Lysis/Binding Solution" on page 13
2. "Homogenize up to 10 mg tissue in 100 µL prepared Lysis/Binding Solution" on page 13
3. "Centrifuge lysate at 1000 x g for 10 min at room temp" on page 14
4. "Transfer 50 µL of lysate to the Processing Plate, add 35 µL100% isopropanol, and shake for 1 min" on page 14

Initial Nucleic Acid Purification



1. "Add 20 µL Bead Mix to each sample; shake for 5 min" on page 14
2. "Magnetically capture the RNA Binding Beads and discard the supernatant" on page 14
3. "Wash with 150 µL Wash Solution 1 for 1 min with shaking" on page 14
4. "Wash with 150 µL Wash Solution 2, and prepare Diluted TURBO™ DNase" on page 14

TURBO DNase™ Treatment and Final RNA Clean-Up

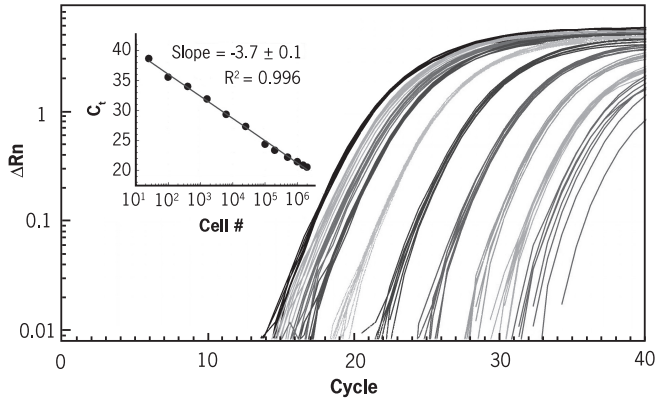


1. "Add 50 µL of Diluted TURBO DNase and shake for 10–15 min at room temp" on page 15
2. "Add 100 µL of RNA Rebinding Solution (isopropanol added) to each sample and shake for 3 min at the maximum speed for larger volumes." on page 15
3. "Magnetically capture the RNA Binding Beads and discard the supernatant" on page 15
4. "Wash twice with 150 µL Wash Solution 2 each time" on page 15
5. "Dry the beads by shaking for 2 min" on page 15
6. "Elute the RNA in 50 µL of Elution Buffer " on page 16

RNA suitable for most downstream applications

The quality and purity of the eluted RNA make it suitable for most common downstream application, such as qRT-PCR, microarray analysis, Northern blotting, and ribonuclease protection assays (RPA). The following figure shows an example of qRT-PCR data that illustrates linear RNA recovery from a broad range of input cell amounts using the MagMAX™-96 Total RNA Isolation Kit.

Figure 2 Linear Recovery of RNA Using the MagMAX™-96 Total RNA Isolation Kit. RNA was isolated from K562 cells in 8 replicate wells using the MagMAX™-96 Total RNA Isolation Kit. Equivalent volumes (4% of eluent) of the recovered RNA were then used in real-time qRT-PCR targeting the human RNA Polymerase II gene (RPII). The CV of C_t values among replicates was less than 3%. The lines represent amplification products from RNA obtained from the following sample sizes (left to right): 2×10^6, 10^6, 2×10^5, 10^5, 2.5×10^4, 6.25×10^4, 1600, 400, 100, and 25 cells.



Extending Utility of the Kit

Recover total nucleic acids

The MagMAX™-96 Total RNA Isolation Kit is designed for RNA isolation; however, by omitting the DNase treatment from the protocol, it can also be used for total nucleic acid isolation. To isolate both RNA and DNA with the kit, omit steps 4. on page 14 through step 3. on page 15, but otherwise follow the protocol for RNA isolation.

Note that excessive amounts of DNA can cause the RNA Binding Beads to clump together, which may result in inefficient elution of DNA/RNA at the end of the procedure. If you experience low yield and/or inconsistent yield, lowering the amount of sample input may improve results.

Kit Components and Storage Conditions

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

Amount		Component	Storage
1		Processing Plate with Lid	room temp
11	mL	Lysis/Binding Solution Concentrate (See section "Sample Homogenization" on page 11 for instructions)	room temp
18	mL	Wash Solution 1 Concentrate (Add 6 mL 100% isopropanol before use)	room temp
55	mL	Wash Solution 2 Concentrate (Add 44 mL 100% ethanol before use)	room temp
12	mL	RNA Rebinding Concentrate (Add 6 mL 100% isopropanol before use)	room temp
10	mL	Elution Buffer	4°C or room temp
6	mL	MagMAX™ TURBO™ DNase Buffer	4°C or room temp
1.1	mL	RNA Binding Beads	4°C†
1.1	mL	Lysis/Binding Enhancer	–20°C
110	µL	TURBO DNase™	–20°C

† Do not freeze the RNA Binding Beads.

Preparation and Sample Homogenization

Equipment and Reagent Preparation

1. RNase precautions

   Lab bench and pipettors

   Before working with RNA, it is always a good idea to clean the lab bench and pipettors with an RNase decontamination solution (e.g., Ambion RNaseZap® Solution, Cat #AM9780).

   Gloves and RNase-free technique

   Wear laboratory gloves for this procedure; they protect you from the reagents, and they protect the RNA from nucleases that are present on skin.

   Use RNase-free pipette tips to handle the kit reagents, and avoid putting used tips into the reagent containers.

2. Determine maximum 96-well plate shaker settings

   For larger volumes

   Place 200 µL water in the wells of a 96-well plate and use it to determine the maximum shaker setting that can be used with your orbital shaker without sample spillage. This maximum shaker speed will be used for most steps of the procedure.

For smaller volumes

Place 50 µL of water in the wells of a 96-well plate and use it to determine the maximum shaker setting that can be used with your orbital shaker without sample spillage. Use this speed for the bead drying and RNA elution steps.

3. Before using the kit, complete Wash Solutions 1 and 2, and RNA Rebinding Solution
   a. Add 6 mL 100% isopropanol to the bottle labeled Wash Solution 1 Concentrate and mix well.

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

   b. Add 44 mL 100% ethanol to the bottle labeled Wash Solution 2 Concentrate and mix well.

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

   c. Add 6 mL 100% isopropanol to the bottle labeled RNA Rebinding Concentrate and mix well.

      The resulting mixture is called RNA Rebinding Solution in these instructions.

   d. Mark the labels of the solutions to indicate that isopropanol or ethanol was added. Store the solutions at room temperature.

4. Prepare Bead Mix

   Each isolation reaction requires 20 µL of Bead Mix. Although the mixture is tested to be 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.

      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.

Sample Homogenization

Sample homogenization instructions optimized for different sample types are listed below. Follow the instructions in this section for cultured mammalian cells, animal tissue samples, or plant tissue samples.

Cultured Mammalian Cells

1. Prepare Lysis/Binding Solution
   - We recommend including 5–10% overage to cover pipetting error.

- Store the Lysis/Binding Solution for cultured cells at room temperature, do not store at 4°C. If the prepared Lysis/Binding Solution is inadvertently stored at 4°C, warm it at room temperature and vortex to dissolve any precipitate that forms—do not heat it.

Per reaction		Entire bottle		Reagent
77	µL	11	mL	Lysis Binding Solution Concentrate
63	µL	9	mL	100% isopropanol
140	µL	20	mL	total Lysis/ Binding Solution

- After assembling the Lysis/Binding Solution, mix thoroughly.

2. Lyse up to 2 x 10^6 cells in 140 µL prepared Lysis/Binding Solution

   Adherent cells: Remove culture media from 2.5 x 10^2 to 2 x 10^6 cells, and immediately add 140 µL Lysis/Binding Solution.

   Suspension cells: Suspend cells in ≤ 30 µL of 1X PBS or in the Ambion RNA*later*® tissue collection: RNA stabilization solution. Transfer cells to wells of a Processing Plate, and add 140 µL Lysis/Binding Solution.

3. Shake for 1 min on an orbital shaker at the maximum speed for larger volumes identified in step 2. on page 10. Cells will lyse during this shaking incubation.

   Adherent cells: Transfer 140 µL cell lysate to wells of the Processing Plate.

   Proceed to step 1. on page 14.

Animal Tissue Samples

1. Prepare Lysis/Binding Solution

   a. Lysis/Binding Solution for processing animal tissue samples must be prepared on the day it will be used. Prepare Lysis/Binding Solution for the number of samples to be processed that day, plus 5–10% overage. Store at room temperature.

Per reaction		Entire bottle		Reagent
100	µL	11	mL	Lysis Binding Solution Concentrate
0.7	µL	77	µL	β-mercaptoethanol (14.3 M)
~100	µL	~11	mL	total Lysis/ Binding Solution

   b. After assembling the Lysis/Binding Solution, mix thoroughly.

2. Homogenize up to 5 mg of tissue in 100 µL prepared Lysis/Binding Solution

   Disrupt and homogenize samples in 100 µL prepared Lysis/Binding Solution using standard homogenization procedures. For most tissues, rotor-stator homogenizers work very well.

   Sample size: For most animal tissue types, 5 mg samples can be processed per reaction. But for tissues that are very high in nucleases, such as spleen and pancreas, use 2.5 mg or less tissue per sample.

Handling fresh tissue: Immediately after dissection, inactivate RNases by any one of the following treatments:

- Freeze rapidly in liquid nitrogen (tissue pieces must be small enough to freeze in a few seconds).
- Submerge in a tissue storage buffer such as Ambion RNA*later* solution.
- Homogenize in prepared Lysis/Binding Solution immediately.

Handling frozen tissue: See section "Handling Frozen Tissue for RNA Isolation" on page 22 for detailed suggestions. Weigh frozen tissue, and if necessary, break it into 2.5–5 mg pieces (keeping tissue completely frozen) and homogenize directly in a rotor-stator homogenizer. Larger pieces of tissue, very hard or fibrous tissues, and tissues with a high RNase content, are typically ground into powder in liquid nitrogen for maximum RNA yield.

Cleanup and decontamination: Wipe the benchtop and all utensils and containers used for tissue handling with a 10% bleach solution to clean and decontaminate them.

3. Add 60 µL100% isopropanol and shake for 1 min
   a. Transfer homogenized sample to wells of the Processing Plate.
   b. Add 60 µL 100% isopropanol to each sample.
   c. Shake for 1 min on an orbital shaker at the maximum speed for larger volumes identified in step 2. on page 10.

      Proceed to step 1. on page 14.

Plant Tissue Samples

1. Prepare Lysis/Binding Solution
   a. Lysis/Binding Solution for processing plant tissue samples must be prepared on the day it will be used. Prepare Lysis/Binding Solution for the number of samples to be processed that day, plus 5–10% overage. Store at room temperature.

Per reaction		Entire bottle		Reagent
90	µL	11	mL	Lysis Binding Solution Concentrate
10	µL	1.22	mL	Plant RNA Isolation Aid (Cat #AM9690)
100	µL	12.22	mL	total Lysis/ Binding Solution

   b. After assembling the Lysis/Binding Solution, mix thoroughly.

2. Homogenize up to 10 mg tissue in 100 µL prepared Lysis/Binding Solution

   Disrupt and homogenize samples in 100 µL prepared Lysis/Binding Solution using standard homogenization procedures for the plant tissue type. Optimal disruption and homogenization procedures vary widely for different plant tissue types, refer to the recent scientific literature for information on disruption of your sample type.

3. Centrifuge lysate at 1000 x g for 10 min at room temp

   Centrifuge homogenized sample at 1000 x g for 10 min at room temp to remove insoluble debris.

4. Transfer 50 µL of lysate to the Processing Plate, add 35 µL100% isopropanol, and shake for 1 min
   a. Transfer 50 µL of homogenized sample to wells of the Processing Plate.
   b. Add 35 µL 100% isopropanol to each sample.
   c. Shake for 1 min on an orbital shaker at the maximum speed for larger volumes identified in step 2. on page 10.

      Proceed to step 1. on page 14.

MagMAX™-96 Total RNA Isolation Kit Protocol

Initial Nucleic Acid Purification

1. Add 20 µL Bead Mix to each sample; shake for 5 min
   a. Gently vortex the Bead Mix to resuspend the magnetic beads. Add 20 µL Bead Mix to each sample.
   b. Shake for 5 min on an orbital shaker at the maximum speed for larger volumes identified in step 2. on page 10 to bind the RNA to the RNA Binding Beads.
2. Magnetically capture the RNA Binding Beads and discard the supernatant
   a. Move the Processing Plate to the magnetic stand to capture the RNA Binding Beads. Leave the plate on the magnetic stand until the beads have completely pelleted. The capture time depends on the magnetic stand used; with the Ambion 96-well Magnetic-Ring Stand, the capture time is ~2–3 min.
   b. Carefully aspirate and discard the supernatant without disturbing the beads, and remove the Processing Plate from the magnetic stand.
3. Wash with 150 µL Wash Solution 1 for 1 min with shaking
   a. Add 150 µL Wash Solution 1 (isopropanol added) to each sample and shake for 1 min at the maximum speed for larger volumes.
   b. Move the Processing Plate to a magnetic stand to capture the RNA Binding Beads. Leave the plate on the magnetic stand until the mixture becomes transparent, indicating that capture is complete. The capture time depends on the magnetic stand used; with the Ambion 96-well Magnetic-Ring Stand, the capture time is ~1–2 min.
   c. Carefully aspirate and discard all supernatant without disturbing the beads, and remove the Processing Plate from the magnetic stand.

      It is critical to remove the Processing Plate from the magnetic stand before the subsequent wash step.
4. Wash with 150 µL Wash Solution 2, and prepare Diluted TURBO™ DNase
   a. Add 150 µL Wash Solution 2 (ethanol added) to each sample and shake for 1 min on an orbital shaker at the maximum speed for larger volumes.
   b. Capture the RNA Binding Beads on a magnetic stand. During this capture step, prepare the diluted TURBO™ DNase as described next.

c. While capturing the RNA Binding Beads, combine the volumes of MagMAX TURBO DNase Buffer with TURBO DNase shown in the table below appropriate for the number of samples being processed plus ~10% overage to cover pipetting error. Mix thoroughly and leave at room temperature until the mixture is needed in step below.

Per reaction		96 rxns (+10%)		Component
49	µL	5.4	mL	MagMAX™ TURBO™ DNase Buffer
1	µL	110	µL	TURBO™ DNase

d. Carefully aspirate and discard the supernatant without disturbing the beads, and remove the Processing Plate from the magnetic stand.

Be sure to remove all Wash Solution 2 before continuing to the TURBO DNase treatment.

e. Proceed immediately to the TURBO DNase treatment and final RNA clean-up.

Turbo DNase Treatment and Final RNA Clean-Up

1. Add 50 µL of Diluted TURBO DNase and shake for 10–15 min at room temp

   When the Diluted TURBO DNase is added to the sample, nucleic acids are released from the RNA Binding Beads, and genomic DNA is degraded.

   a. Add 50 µL Diluted TURBO DNase to each sample.

   b. Shake the plate on an orbital shaker for 10–15 min at room temp at the maximum speed for larger volumes identified in step 2. on page 10.

2. Add 100 µL of RNA Rebinding Solution (isopropanol added) to each sample and shake for 3 min at the maximum speed for larger volumes.

   In this step, the RNA is bound to the RNA Binding Beads again.

3. Magnetically capture the RNA Binding Beads and discard the supernatant

   a. Capture the RNA Binding Beads on a magnetic stand.

   b. Carefully aspirate and discard the supernatant without disturbing the beads, and remove the Processing Plate from the magnetic stand.

4. Wash twice with 150 µL Wash Solution 2 each time

   a. Add 150 µL Wash Solution 2 to each sample and shake for 1 min on an orbital shaker at the maximum speed for larger volumes.

   b. Capture the RNA Binding Beads on a magnetic stand.

   c. Carefully aspirate and discard the supernatant without disturbing the beads, and remove the Processing Plate from the magnetic stand.

   d. Repeat wash for a second time.

5. Dry the beads by shaking for 2 min

   Move the Processing Plate to the shaker and shake vigorously for 2 min at the maximum speed for lower volumes identified in step 2. on page 10.

   This dries the beads, removing residual ethanol which otherwise could interfere with downstream applications.

6. Elute the RNA in 50 µL of Elution Buffer

   a. Add 50 µL Elution Buffer to each sample and shake vigorously for 3 min at the maximum speed for lower volumes.

      Note: The elution volume is somewhat flexible; RNA can be eluted as little as 20 µL, or in >50 µL to achieve the desired final RNA concentration. The volume of Elution Buffer supplied with the kit is enough for 96 samples at 100 µL each.

   b. Capture the RNA Binding Beads on a magnetic stand. 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.

Assessing RNA Yield and Purity

RNA yield

- Spectrophotometry

  The concentration of an RNA solution can be determined by measuring its absorbance at 260 nm (A_{260}). We recommend using the NanoDrop® 1000A Spectrophotometer because it is extremely quick and easy to use; just measure 1–2 µL of the RNA sample directly.

  Alternatively, the RNA concentration can be determined by diluting an aliquot of the preparation in TE (10 mM Tris-HCl, pH 8; 1 mM EDTA) and reading the absorbance in a traditional spectrophotometer at 260 nm. To determine the RNA concentration in µg/mL, multiply the A_{260} by the dilution factor and the extinction coefficient (1 A_{260} = 40 µg RNA/mL).

  $$A_{260} \text{ X dilution factor X } 40 = \mu g \text{ RNA/mL}$$

  Be aware that any contaminating DNA in the RNA prep will lead to an overestimation of yield, since all nucleic acids absorb at 260 nm.

- Fluorometry

  If a fluorometer or a fluorescence microplate reader is available, Molecular Probes' RiboGreen® fluorescence-based assay for RNA quantitation is a convenient and sensitive way to measure RNA concentration. Follow the manufacturer's instructions for using RiboGreen.

RNA quality

- Microfluidic analysis

  The Agilent® 2100 Bioanalyzer™ instrument with Caliper's RNA LabChip® Kits provides better quantitative data than conventional gel analysis for characterizing RNA. When used with Ambion RNA 6000 Ladder (Cat #AM7152), this system can provide a fast and accurate size distribution profile of RNA samples. Follow the manufacturer's instructions for performing the assay.

  The 28S to 18S rRNA ratio is often used as an indicator of RNA integrity. Total RNA isolated from fresh and frozen mammalian tissues using this kit usually has a 28S to 18S rRNA ratio of >1.2.

  Using a Bioanalyzer™ instrument, the RIN (RNA Integrity Number) can be calculated to further evaluate RNA integrity. A new metric developed by Agilent, the RIN analyzes information from both rRNA bands, as well as information contained outside the rRNA peaks (potential degradation products) to provide a fuller picture of RNA degradation states. Search for "RIN" at Agilent's website for information: www.chem.agilent.com

- Spectrophotometry

  An effective measure of RNA purity is the ratio of absorbance readings at 260 and 280 nm. The total RNA isolated with this kit should have an A_{260}/A_{280} ratio of 1.8–2.1. However, RNA with absorbance ratios outside of this range may still function well for qRT-PCR or other amplification-based downstream applications.

Troubleshooting

DNA Contamination

Too much sample input

If sample input was too high (>2 x 10^6 cells, >5 mg animal tissue, or >5–10 mg plant tissue), the DNA digestion step may not be effective. To avoid DNA contamination, either reduce sample size, or include an additional DNase treatment step after RNA isolation (e.g., using the Ambion TURBO™ DNA-*free*™ Kit, Cat #AM1907).

Ambient temperature is too cool

Since the DNA digestion is done at room temperature, it is important that the ambient temperature in your lab be 20–25°C. If your lab is colder than 20°C, incubate the digestion in a 25°C incubator.

Well-to-Well Variation in RNA Yield

The RNA yield should be fairly uniform between wells of a 96-well plate containing the same number of cells. If a large variation in RNA yield between wells is observed, consider the following:

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. We have also found that using the Ambion 96-well Magnetic-Ring Stand (Cat #AM10050) results in RNA Binding Bead pellets that are significantly easier to resuspend than pellets captured using other magnetic stands.

a. Make sure the RNA Binding Beads are fully resuspended before pipetting them into the Processing Plate at the start of the procedure.

b. For efficient elution of RNA from the RNA Binding Beads at the end of the procedure, make sure the beads are fully dispersed in Elution Buffer.

   If the RNA Binding Beads aggregate or fail to disperse during the final RNA elution step, it may improve RNA yield to place the Processing Plate in a 70°C incubator for 5 min and to repeat the 3 min shaking incubation before capturing the beads.

   In subsequent experiments using sample types with bead clumping problems, you can preheat the Elution Buffer to 70–80°C before adding it to the samples in step 6. on page 16 to facilitate dispersion of the beads.

c. Do not overdry the beads before eluting. If the beads were inadvertently overdried, extend the shaking time in step 6. on page 16 to 10 min to rehydrate the beads.

RNA Binding Beads were unintentionally lost

Since the basis of this procedure is to immobilize RNA on RNA Binding Beads, any loss of beads during the procedure will result in loss of RNA. Avoid aspirating RNA Binding Beads when removing supernatant from the captured beads. To determine whether RNA Binding Beads have been inadvertently aspirated with supernatant, it may be helpful to collect all supernatants in a single reservoir. Observe the color of the collected supernatant, if RNA Binding Beads are in the supernatant, they will tint the solution light brown.

To prevent aspiration of RNA Binding Beads in subsequent experiments, observe the following precautions:

- Use sufficient magnetic capture time.
- Aspirate supernatant slowly.
- Keep pipet tip openings away from the captured RNA Binding Beads when aspirating supernatant.

A Materials not included with the Kit

Required Materials Not Provided With the Kit

Reagents/equipment

- 100% ethanol, ACS grade or higher quality
- 100% isopropanol, ACS grade or higher quality
- To use the kit manually, you will need an orbital shaker for 96-well plates such as the Barnstead/Lab-Line Titer Plate Shaker (available from VWR and Fisher Scientific).
- Magnetic stand for 96-well plates: We recommend either of the Ambion 96-well magnetic stands (Part no. #AM10050, AM10027) for their high strength magnets and quality design (See "Related Products Available from Life Technologies" on page 20).
- If you process fewer than 96 samples at a time, you will need additional polystyrene U bottom 96-well plates and lids.

RNA isolation from animal tissue samples

- 14.3 M β-mercaptoethanol (β-ME)

RNA isolation from plant tissue samples

- Plant Isolation Aid (Cat #AM9690)

Automation equipment

For completely automated RNA isolation with the MagMAX™-96 Total RNA Isolation Kit, the robotic liquid handler must have the following features:

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

Related Products Available from Life Technologies

96-well Magnetic-Ring Stand Part no. AM10050	The Ambion 96-well Magnetic-Ring Stand features 96 powerful ring-shaped magnets arranged to cradle each well of a 96-well plate for quick, thorough bead capture. Captured magnetic beads form evenly distributed donut-shaped pellets with a large hole in the center. This capture pattern facilitates both supernatant removal and subsequent bead resuspension. The stand is suitable for high throughput applications conducted with multichannel pipettors or with robotic liquid handlers. However, because the pellets will be evenly distributed around the edge of the wells, it may require practice for efficient manual removal of supernatants.
Magnetic Stand-96 Part no. AM10027	The Ambion Magnetic Stand-96 has powerful magnets positioned to capture beads to one side of the well. This capture pattern makes it very easy to remove supernatants manually without disturbing the beads, and therefore may be preferred by beginning users. In some applications, however, pellets formed with the Magnetic Stand-96 may be difficult to resuspend. If this occurs, we recommend the 96-well Magnetic-Ring Stand (Cat #AM10050).
Plant RNA Isolation Aid Part no. AM9690	The Plant RNA Isolation Aid contains polyvinylpyrrolidone (PVP) to selectively remove polysaccharides and polyphenolics from plant lysates during RNA isolation. It is compatible with most RNA isolation procedures that use chaotropic salt-based lysis solutions.
RNAlater® Solution Part nos. 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.
RNAlater®-ICE Solution Part nos. AM7030, AM7031	RNA*later*-ICE Frozen Tissue Transition Solution is designed to make it easier to process frozen tissue samples for RNA isolation. Simply drop frozen tissues into RNA*later*-ICE Solution and store overnight at –20°C. Once tissues are treated they can be easily processed using standard RNA isolation procedures.
RNaseZap® Solution Part nos. AM9780, AM9782, AM9784	RNaseZap 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 RNaseZap Solution.
MessageAmp™ II aRNA Amplification Kits	Life Technologies offers a full line of MessageAmp II Kits tailored for different array analysis applications. The MessageAmp II Kit offers maximum flexibility; samples can be amplified using either single- or double-round amplification, and the reagent cocktails are configured to accommodate modification. For arrays requiring biotin-labeled samples, Life Technologies offers the MessageAmp II-Biotin *Enhanced* Single Round aRNA Amplification Kit. For preparation of fluorescently-labeled samples, we recommend the Amino Allyl MessageAmp II Kits which are available with and without Cy™3 and Cy5. Bacterial RNA can be amplified using the MessageAmp II Bacteria RNA Amplification Kit. We also offer the MessageAmp II-96 and Amino Allyl MessageAmp II-96 aRNA Amplification Kits for high throughput applications.
MessageSensor™ RT Kit Part no. AM1745	The MessageSensor RT Kit for one-step qRT-PCR includes an optimized set of reagents for exceptionally sensitive reverse transcription. The kit is designed to be used for single-tube amplification of mRNA using either real-time or end-point amplification strategies.

B

Additional Procedures

KingFisher MagMAX™-96 Total RNA Isolation Kit Protocol Overview

The MagMAX-96 Total RNA Isolation protocol can be adapted for use with Thermo Electron's KingFisher (for 1–24 samples per run) and KingFisher 96 (for 1–96 samples per run) Magnetic Particle Processors.

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

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

Row / Plate		Volume		Reagent(s)
A	For Cultured Cells	140	µL	Lysis/Binding Solution (isopropanol added)
		≤30	µL	Sample
		20	µL	**Bead Mix**
		190	µL	total volume
	For Animal Tissues	100	µL	Tissue homogenate in Lysis Binding Solution
		60	µL	Isopropanol
		20	µL	**Bead Mix**
		180	µL	total volume
	For Plant Tissues	50	µL	Tissue homogenate in Lysis/Binding Solution
		35	µL	Isopropanol
		20	µL	**Bead Mix**
		110	µL	total volume
B		150	µL	Wash Solution 1
C		150	µL	Wash Solution 2
D		50	µL	Diluted TURBO DNase
E		150	µL	Wash Solution 2
F		150	µL	Wash Solution 2
G		50	µL	Elution Buffer

2. Choose the MagMAX™ Total program using the arrow keys and start the program by pressing the START button. The approximately 25 min process is described below.
3. Total RNA is bound to RNA Binding Beads in row A (plate A the KingFisher 96) containing sample, Lysis/Binding Solution, and Bead Resuspension Mix.
4. The RNA Binding Beads are collected and released into the first Wash 1 Solution in row B (plate B).
5. The RNA Binding Beads are collected and released into the first Wash 2 Solution in row C (plate C).
6. The RNA Binding Beads are collected and released into the diluted TURBO DNase in row D (plate D).
7. The machine pauses and 100 µL of re-binding solution is added to row D (plate D) by the user to bind the RNA. Press the START button to continue.
8. The RNA Binding Beads are collected and released into the second Wash 2 Solution in row E (plate E).
9. The RNA Binding Beads are collected and lifted outside the wells of row F (plate F) to dry for 1 min.
10. The RNA Binding Beads are released into Elution Buffer in row G (plate G).
11. The used RNA Binding Beads are collected and returned to row B (plate B leaving RNA in elution Buffer in row G [plate G]).

Handling Frozen Tissue for RNA Isolation

Keep samples at –80°C until you are ready to begin

It is very important to maintain frozen tissue either at –80°C for long term storage, or on dry ice for short periods of time to allow for tissue handling and partitioning. RNA in tissue that has undergone a freeze/thaw cycle without protection by lysis reagents or RNA*later* will be degraded.

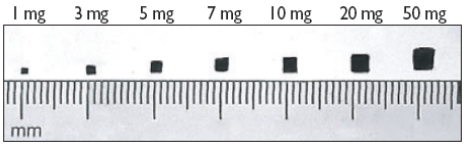
Recommendations for keeping samples frozen during partitioning

- Prepare a *closed container* (such as an ice chest) containing enough dry ice to create a cold vapor environment.
- To further safeguard against defrosting of small tissue fragments, prechill the container (such as a small tip box or a mortar and pestle decontaminated with RNaseZap) that will be used to hold the frozen tissue over dry ice during partitioning.
- Plan to use either a sharp scalpel with a handle and a nonflexible metal blade or a mortar and pestle with liquid nitrogen to fragment frozen tissue into 2.5–5 mg pieces. You will also need a pair of forceps manufactured with small 'grip' teeth near the point to grasp frozen tissue firmly. Decontaminate containers and tools with RNase*Zap*, and pre-chill in dry ice.

Instructions for partitioning tissue samples into 2.5–5 mg pieces

There are two suggested strategies for breaking frozen tissue samples into 2.5–5 mg pieces: cutting the tissue with a scalpel or freezing it in liquid nitrogen and breaking it by gently crushing in a mortar and pestle. We present detailed instructions for both of these methods below. The most important aspect of either procedure, however, is to work quickly and to keep frozen samples frozen completely. Note that a 5 mg tissue fragment is quite small—roughly the size of a 2 mm cube. The figure below can be used to help estimate the weight of tissue fragments.

Figure 3 Estimating the Mass of Small Tissue Samples. Approximately 1–2 mm thick pieces of mouse liver tissue with the indicated weights were placed on a ruler, and photographed. Most other soft tissues are of similar density.



Use a scalpel to chop tissue into ≤2.5–5 mg pieces

Working quickly in a prechilled container that is resting on dry ice in an ice chest, slice or carve the frozen tissue into pieces (see the above figure as a guide). Partitioning frozen tissue into milligram pieces can be a challenge; we recommend the following technique to carve tissue effectively and rapidly. Grasp the frozen tissue firmly with chilled forceps. Position the scalpel tip on the surface of the pre-chilled container at a ~45 degree angle just in front of the tissue. Working at the edge of the tissue mass, press down firmly in a chopping motion without raising the tip of the scalpel, and chisel off a small fragment of tissue. Continue this chisel-like action until you have more tissue fragments than you need. Keep all the tissue fragments in the container resting on dry ice until you are ready to homogenize the tissue. (Keep the container closed when it is not in use.)

Break tissue by freezing in liquid nitrogen and gently crushing

Set up an ice chest with dry ice and a mortar and pestle that has been treated with RNase*Zap*. Fill the mortar with liquid nitrogen to a depth of 0.5–1 cm and drop in the tissue sample (tissue that was stored in foil can remain in the foil or can be removed from the foil). Allow the tissue to equilibrate to the temperature of the liquid nitrogen for several seconds, and add more liquid nitrogen if necessary so that the tissue is about half immersed. Next, gently press on the tissue with the pestle to partially crush it, breaking it into 2.5–5 mg pieces in the presence of liquid nitrogen. Keep the tissue fragments frozen in the container resting on dry ice until you are ready to homogenize the tissue.

Estimate the weight of tissue fragments

Weighing tissue is the most accurate way to quantify the amount of starting material, however, it is simply not practical for most experiments. Instead, use Figure 3 as a guideline to estimate appropriately-sized tissue fragments.

C

Safety

General 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, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

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.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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/**

Bibliography

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.

Documentation and Support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/sds**

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

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

www.lifetechnologies.com

life technologies™