invitrogen

MagMAX™-96 for Microarrays Total RNA Isolation Kit

USER GUIDE

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Revision H

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

ThermoFisher SCIENTIFIC

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Revision	Date	Description
H	12 March 2018	Rebranding and streamlining of the protocol
G	November 2011	Baseline for revision

Contents

MagMAX™-96 for microarrays total RNA isolation kit

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 in this document.

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

Purpose

The *MagMAX™-96 for Microarrays 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 for descriptions of the symbols.

Introduction

Product description and procedure overview

The MagMAX™-96 for Microarrays Total RNA Isolation Kit is designed for rapid, high throughput isolation of total RNA from mammalian cells and tissues in 96-well plates. The procedure employs the robust and reliable lysis/denaturant, TRI Reagent™ solution, and the MagMAX™ magnetic bead-based RNA purification technology. 96 samples can be processed at once with the MagMAX™-96 for Microarrays Kit, however, it can also be used to efficiently isolate RNA from fewer than 96 samples. The MagMAX™-96 for Microarrays Kit is optimized for use with either manual multichannel pipettors or with robotic liquid handlers.

TRI Reagent™ solution and MagMAX™-96 technology

TRI Reagent™ solution lyses cells and facilitates sample homogenization while denaturing nucleases to maintain the integrity of RNA. In the MagMAX™-96 for Microarrays Kit, TRI Reagent™ solution is used in combination with our MagMAX™ technology. MagMAX™ magnetic beads bind RNA more efficiently and reproducibly than glass fiber filters, and they are quick and simple to use. By combining TRI Reagent™ solution and MagMAX™ technology, you get streamlined RNA purification, even in high throughput format, without sacrificing RNA quantity or quality.

One kit: two alternative procedures

The procedure is fast (<1 hr), simple, and well-suited for automation. First, mammalian cultured cells or tissues samples are homogenized in TRI Reagent™ solution, a monophasic solution containing phenol and guanidine thiocyanate. This rapidly lyses cells and inactivates nucleases. Once the tissue is homogenized in TRI Reagent™ solution, either of two alternative procedures can be followed for RNA isolation: the Spin Procedure and the No-Spin Procedure.

Spin Procedure	No-Spin Procedure
Better for difficult samples such as spleen and pancreas, and for RNA*later*™+ICE reagent-treated samples	Appropriate for low fat tissues and low cellular content samples
Fewer steps: faster if only a few samples are processed	No phase separation
Compatible with tissue storage in RNA*later* reagent	Easy to automate
No DNase treatment needed	
More tissue can be processed per sample	

The ***Spin Procedure*** begins with the addition of bromochloropropane (BCP) and centrifugation to separate the aqueous and organic phases. The aqueous phase, containing partially purified RNA is then transferred to the wells of a 96-well plate. The RNA is then further purified using a simple magnetic bead binding and washing procedure; no DNase treatment is required. Purified RNA is eluted in 50–100 µL of low salt buffer.

Alternatively, samples can be processed using the ***No-Spin Procedure*** which starts with an initial nucleic acid purification in which magnetic beads are added directly to the homogenized sample to bind nucleic acids. Using magnetic capture, the beads and bound nucleic acids are then subjected to three rapid washing steps to remove proteins and salt. In the next phase of the procedure, samples are treated with TURBO™ DNase to remove genomic DNA, and the total RNA is rebound to the magnetic beads for two final washing steps. The purified RNA is eluted in 50 µL of low salt buffer.

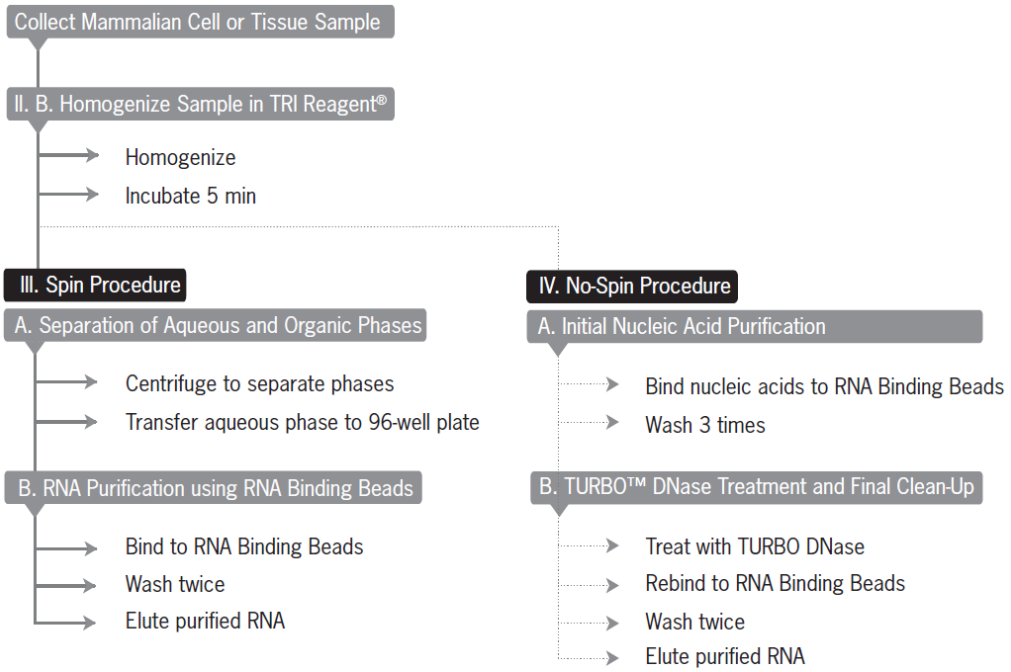


Figure 1 MagMAX™-96 for microarrays kit procedure overview

High yield and high quality RNA from both procedures

Both the Spin and the No-Spin MagMAX™-96 for Microarrays Kit procedures provide high yields of pure, intact RNA that can be used directly for quantitative reverse transcriptase PCR (qRT-PCR) and microarray analysis. The following figure shows RNA yield and quality data that illustrates the performance of the MagMAX™-96 for Microarrays RNA Isolation Kit. In this experiment, highly intact RNA purified from frozen mouse liver, consistently yielded 28S/18S ribosomal RNA (rRNA) ratios of 1.1–1.4.

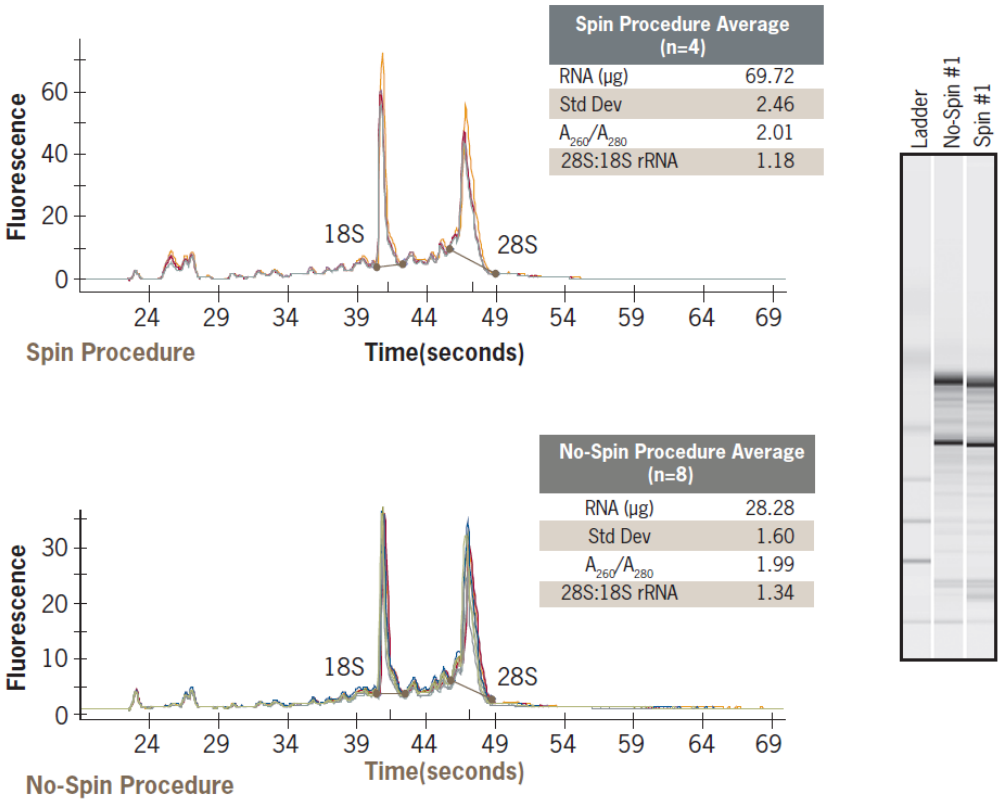


Figure 2 Consistent yield, purity, and integrity of RNA isolated with the MagMAX™-96 for microarrays kit. RNA was isolated from frozen mouse liver with the MagMAX™-96 for microarrays kit. Tissue homogenates were processed in quadruplets or octuplets using the spin procedure (homogenate derived from 10 mg tissue per sample) or the No-Spin procedure (homogenate derived from 5 mg tissue per sample), respectively. Purified RNA (2 µL) was quantified using a NanoDrop™ spectrophotometer. The ratio of 28S to 18S rRNA was obtained by analyzing purified RNA (1 µL) using an RNA LabChip™ Kit and the Agilent™ 2100 Bioanalyzer™ instrument.

Kit components and storage conditions

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

Amount	Component	Storage
1 each	Processing Plate & Lid	room temp
12 mL	Lysis/Binding Solution Concentrate Add 6 mL 100% isopropanol before use.	room temp
18 mL	Wash Solution 1 Concentrate Add 6 mL 100% isopropanol before use.	room temp
72.5 mL	Wash Solution 2 Concentrate Add 58 mL 100% ethanol before use.	room temp
14 mL	Elution Buffer	room temp
6 mL	MagMAX™ TURBO™ DNase Buffer	4°C or room temp
100 mL	TRI Reagent™ solution (four 25 mL bottles)	4°C
1.1 mL	RNA Binding Beads	4°C[1]
1.1 mL	Lysis/Binding Enhancer	–20°C
215 µL	TURBO™ DNase (10 U/µL)	–20°C

[1] Do not freeze the RNA Binding Beads.

Experimental setup and sample homogenization

Reagent and equipment preparation

RNase precautions:

- Lab bench and pipettors
  - Before working with RNA, clean the lab bench and pipettors with an RNase decontamination solution (e.g., RNase*Zap*™ Solution).
- Gloves and RNase-free technique
  - Wear laboratory gloves; 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.

1. Determine maximum 96-well plate shaker settings.

   For larger volumes

   Place 180 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 100 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.

2. Before using the kit, complete the Lysis/Binding Solution, and Wash Solutions 1 and 2.

   a. Add 6 mL 100% isopropanol to the bottle labeled Lysis/Binding Solution Concentrate and mix well.

      The mixture is called Lysis/Binding Solution in these instructions.

   b. 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.

   c. Add 58 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.

   d. Mark the labels of the solutions to indicate that the isopropanol or ethanol was added.

      Store the solutions at room temperature.

Sample homogenization

1. Homogenize samples in TRI Reagent™

   Separate instructions are provided for sample homogenization of tissue and for cultured cells below.

The maximum amount of sample homogenate that can be used in the MagMAX™-96 for Microarrays procedure depends on which method is followed for the RNA isolation.

- For the ***Spin*** procedure, a maximum of 100 µL of aqueous phase after centrifugation can be used per RNA isolation reaction; we recommend preparing ~500 µL–1 mL of sample homogenate so that you can recover ≥100 µL of aqueous phase.
- For the ***No-Spin*** procedure, a maximum of 100 µL of sample homogenate can be used per RNA isolation reaction.

For samples that are not limited in supply, it is often easier to prepare more homogenate than can be used in a single RNA isolation reaction; the volume of TRI Reagent™ supplied with the kit is sufficient for 96 samples using 1 mL per sample.

- **Homogenize tissue in 10–40 volumes of TRI Reagent™**
  a. Tissue handling instructions

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

     a. Homogenize in TRI Reagent™ immediately (follow the instructions in the next step below).
     b. Freeze rapidly in liquid nitrogen (tissue pieces must be small enough to freeze in a few seconds).
     c. Submerge in a tissue storage buffer such as Ambion™ RNA*later* Solution (Spin procedure ***only;*** samples stored in RNA*later* reagent cannot be used in the No-Spin procedure).

     Handling frozen tissue: Weigh frozen tissue, and if necessary, break it into pieces smaller than ~50 mg (keeping tissue completely frozen) and homogenize directly in TRI Reagent™ solution. Larger pieces of tissue, very hard or fibrous tissues, and tissues with a high RNase content, must typically be ground to a powder in liquid nitrogen for maximum RNA yield.

  b. Tissue homogenization instructions

     Homogenize samples in TRI Reagent™ using standard homogenization procedures. For most tissues, rotor-stator homogenizers work very well.

     Spin procedure: Homogenize samples in 10–20 volumes TRI Reagent™ (e.g., 1 mL TRI Reagent™ per 50–100 mg tissue).

     No-Spin procedure: For most sample types 20 volumes of TRI Reagent™ solution is appropriate (e.g., 1 mL TRI Reagent™ solution per 50 mg tissue), but for tissues that are very high in nucleases, such as spleen and pancreas, homogenize in 40 volumes of TRI Reagent™ solution (e.g., 1 mL TRI Reagent™ per 25 mg tissue).

- **Homogenize cultured cells in 1 mL TRI Reagent™ per 10 cm^2 culture dish area, or per 5 x 10^6 cells**

  Do not wash cells before lysing with TRI Reagent™ as this may contribute to mRNA degradation.

  Cells grown in monolayer: Pour off media, add 1 mL of TRI Reagent™ per 10 cm^2 of culture dish area, and pass the mixture through a pipette several times to lyse cells and homogenize the sample (lyse directly in the culture

dish). Use the area of the culture dish, rather than the cell number, to determine the volume of TRI Reagent™ for lysis.

Cells grown in suspension: Pellet cells, then lyse in 1 mL of TRI Reagent™ per 5 x 10^6 animal, plant, or yeast cells by repeated pipetting or vortexing.

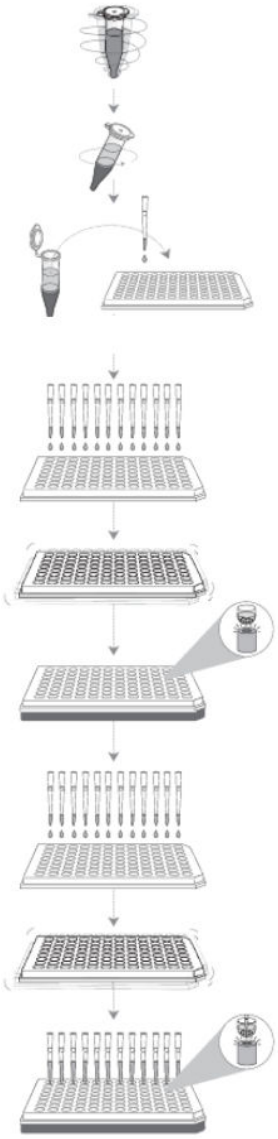
2. Incubate the homogenate for 5 min at room temp

   Incubate homogenates from both tissue samples and cell cultures for 5 min at room temp. This incubation allows nucleoprotein complexes to completely dissociate.

STOPPING POINT Homogenized samples can be stored at –70°C for at least one month.

Continue to either section “Spin procedure“ on page 13 or to section “No-spin procedure“ on page 16.

Spin procedure



Separation of aqueous and organic phases

1. Mix homogenate with 0.1 volumes BCP and leave at room temp for 5 min.
   a. Transfer homogenized sample to a 1.5 mL microcentrifuge tube. Add 0.1 volumes of BCP (e.g., add 100 µL BCP to 1 mL of homogenate), and cap the tube securely.
   b. Vortex at moderate speed for 5–10 seconds.
   c. Store the mixture at room temperature for 5 min.

2. Centrifuge at 12,000 x g for 10 min at 4°C.

   Centrifuge at 12,000 x g for 10 min at 4°C to separate the sample mixture into three phases: phenol-BCP on the bottom (red), interphase in the center, and aqueous phase on the top (colorless). RNA is in the aqueous phase, while DNA and proteins are in the interphase and organic phase (phenol-BCP).

3. Transfer 100 µL aqueous phase to the Processing Plate.

   Transfer 100 µL of the aqueous phase to a well of the 96-well Processing Plate and continue the procedure.

   You can discard the tube after removing the aqueous phase.

RNA purification using RNA binding beads

1. Add 50 µL of 100% isopropanol and shake for 1 min.
   a. Add 50 µL of 100% isopropanol to each sample.
   b. Shake the Processing Plate for 1 min on an orbital shaker at the maximum speed for larger volumes identified step 2 on page 10.

2. Add 10 µL of RNA Binding Beads and shake for 3 min.
   a. Vortex the RNA Binding Beads at moderate speed to create a uniform suspension before pipetting.
   b. Add 10 µL of RNA Binding Beads to each sample.
   c. Shake the plate for 3 min on an orbital shaker at the maximum speed for larger volumes.

3. Magnetically capture the RNA Binding Beads and discard the supernatant.
   a. 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. Using the Ambion™ 96-Well Magnetic-Ring Stand, the capture time is ~1–2 min.
   b. Carefully aspirate and discard the supernatant without disturbing the beads, and remove the Processing Plate from the magnetic stand.

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

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 as in the previous step.
   c. Carefully aspirate and discard the supernatant without disturbing the beads, and remove the Processing Plate from the magnetic stand.
   d. Repeat above steps with a second 150 µL of Wash Solution 2.

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.

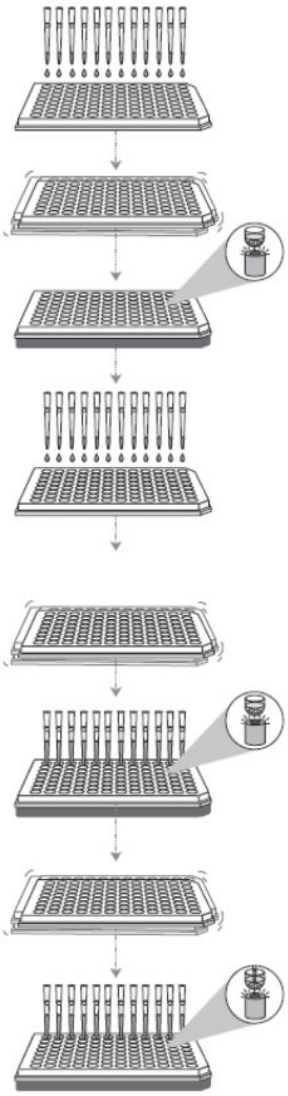
   a. Add 50 µL Elution Buffer to each sample and shake vigorously for 3 min at the maximum speed for lower volumes.

      Note: The elution volume is somewhat flexible; RNA can be eluted 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.

No-spin procedure



Initial Nucleic Acid Purification

1. Mix 100 µL homogenate and 10 µL BCP and shake for 1 min
2. Add 50 µL of 100% isopropanol and shake for 1 min
3. Add 10 µL of RNA Binding Beads and shake for 3 min
4. Magnetically capture the RNA Binding Beads, discard the supernatant, and prepare mixture of Wash Solution 1 and Lysis/Binding Enhancer
5. Wash with 150 µL Wash Solution 1 for 3 min with shaking
6. Wash twice with 150 µL Wash Solution 2, and prepare Diluted TURBO DNase

TURBO™ DNase Treatment and Final Clean-Up

1. Add 50 µL of Diluted TURBO DNaseand shake for 10 min
2. Add 100 µL Lysis/Binding Solution and shake for 3 min to rebind the RNA
3. Magnetically capture the RNA Binding Beads and discard the supernatant
4. Wash twice with 150 µL Wash Solution 2 each time
5. Dry the beads by shaking for 2 min
6. Elute the RNA in 50 µL Elution Buffer

Initial nucleic acid purification

1. Mix 100 µL homogenate and 10 µL BCP and shake for 1 min.
   a. For each sample to be processed, place 10 µL of BCP into a well of the 96-well Processing Plate.
   b. Add 100 µL of homogenized sample into each well containing BCP.
   c. Shake the Processing Plate for 1 min on an orbital shaker at the maximum speed for larger volumes identified in step 2 on page 10.
2. Add 50 µL of 100% isopropanol and shake for 1 min.
   a. Add 50 µL of 100% isopropanol to each sample.
   b. Shake the Processing Plate for 1 min on an orbital shaker at the maximum speed for larger volumes.

3. Add 10 µL of RNA Binding Beads and shake for 3 min.
   a. Vortex the RNA Binding Beads at moderate speed to create a uniform suspension before pipetting.
   b. Add 10 µL of RNA Binding Beads to each sample.
   c. Shake the plate for 3 min on an orbital shaker at the maximum speed for larger volumes.
4. Magnetically capture the RNA Binding Beads, discard the supernatant, and prepare mixture of Wash Solution 1 and Lysis/Binding Enhancer.
   a. Move the Processing Plate to a 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 ~1–2 min.
   b. While capturing the RNA Binding Beads, prepare 150 µL of Wash Solution 1 with Lysis/Binding Enhancer for each RNA isolation reaction according to the table below. We recommend including ~10% overage to cover pipetting error. Mix thoroughly by gently vortexing or pietting up and down a few times.

Component	per rxn		~100 rxns	
Wash Solution 1[1]	140	µL	15.4	mL
Lysis/Binding Enhancer	10	µL	1.1	mL

[1] We recommend preparing this solution just before use. Use it no more than 1 hr after preparing it.

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

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

5. Wash with 150 µL Wash Solution 1 for 3 min with shaking.
   a. Add 150 µL Wash Solution 1 with Lysis/Binding Enhancer to each sample and shake for 3 min on an orbital shaker at the maximum speed for larger volumes.
   b. Capture the RNA Binding Beads on a magnetic stand as described previously.
   c. Carefully aspirate and discard all supernatant without disturbing the beads, and remove the Processing Plate from the magnetic stand.

IMPORTANT! Complete removal of the supernatant is critical.

6. Wash twice with 150 µL Wash Solution 2, and prepare Diluted TURBO DNase™.
   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. During this capture step, prepare the diluted TURBO DNase™ as described in step e on page 18.

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

d. Repeat above steps with a second 150 µL of Wash Solution 2. Be sure to remove all Wash Solution 2 before continuing immediately to the TURBO DNase™ treatment.

e. 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 Diluted TURBO DNase™ is needed in the steps below.

Component	per reaction		~100 reactions	
MagMAX™ TURBO™ DNase Buffer	48	µL	5.3	mL
TURBO™ DNase	2	µL	220	µL

TURBO™ DNase treatment and final Clean-Up

1. Add 50 µL of Diluted TURBO DNase™ and shake for 10 min.

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

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

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

   IMPORTANT! Do not exceed 10 min for the TURBO DNase™ treatment.

2. Add 100 µL Lysis/Binding Solution and shake for 3 min to rebind the RNA.

   Add 100 µL of Lysis/Binding Solution to each sample and shake for 3 min on an orbital shaker 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 as described previously.

   Using the 96-Well Magnetic-Ring Stand, the capture time is ~1 min.

   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 as in the previous steps.

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

d. Repeat above steps to wash with a second 150 µL of Wash Solution 2.

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 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 the RNA and troubleshooting

Assessing RNA yield and purity

RNA yield

- Spectrophotometry

  The concentration of an RNA solution can be determined by measuring its absorbance at 260 nm. Life Technologies™ scientists 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} X dilution factor X 40 = µg 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 manufacturers instructions for using RiboGreen™.

RNA quality

- Microfluidic analysis

  The Agilent™ 2100 Bioanalyzer™ instrument with Caliper™'s RNA LabChip™ Kits provides better qualitative data than conventional gel analysis for characterizing RNA. When used with the Ambion™ RNA 6000 Ladder (Part no. 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:

  http://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 poor RNA yield or integrity

Well-to-well variation in RNA yield

The total RNA yield should be fairly uniform between wells of a 96-well plate with the same sample type; however, RNA recovery from different samples types may vary considerably. The following troubleshooting suggestions may be helpful if large variations in RNA yield from the same sample type are observed.

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 (68°F).

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

2. If RNA integrity is poor or yield is lower than expected, make sure that the RNA Binding Beads are completely resuspended during the TURBO DNase™ treatment. If necessary, pipet the solution up and down to thoroughly resuspend the solution.

3. 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 to facilitate dispersion of the beads.

4. Do not overdry the beads before eluting. If the beads were inadvertently overdried, extend the shaking time 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.

Troubleshooting DNA contamination

With the No-Spin procedure

- Try the Spin Procedure

  If RNA obtained using the No-Spin procedure contains more genomic DNA contamination than your downstream assay can tolerate, you may want to try the Spin procedure with that sample type in subsequent experiments. We also recommend the Ambion™ TURBO™ DNA-*free*™ Kit (Part no. AM1907) for removal of genomic DNA from RNA samples.

- Mix the RNA Binding Beads before shaking

  If RNA Binding Beads aggregate or fail to disperse in section “Initial nucleic acid purification“ on page 16, it may improve DNA digestion to mix the RNA Binding Beads with the sample by pipetting up and down once or twice before the shaking incubation.

With the spin procedure

- Separate the aqueous and organic phases by centrifuging at 4°C

  The Spin procedure includes a centrifugation at 4°C to separate homogenized samples into three distinct phases. It is important to perform the centrifugation at 4°C for maximum separation of the DNA-containing interphase, centrifuging at

room temperature typically increases DNA contamination of the aqueous phase which contains the RNA.

- Avoid touching the interphase when collecting the aqueous phase
  It is important to avoid touching the interphase when collecting the aqueous phase after the centrifugation.

Troubleshooting impurities that inhibit downstream applications

Most impurities will cause a shift in UV absorbance that can be seen by comparing a sample's UV absorbance spectrum to that of a control RNA. Any distortion in the shape of UV spectrum indicates that there are impurities in the eluted RNA. For example, protein absorbs at 280 nm, which can result in a low $\mathbf{A}_{260}/\mathbf{A}_{280}$ ratio. Salt contamination may cause a peak at 230 nm.

RNA isolation reagent carryover

The Lysis/Binding and Wash Solutions contain significant amounts of proteins and salts. To avoid protein and salt carryover, remove supernatants from captured RNA Binding Beads thoroughly.

Troubleshooting RNA binding bead carryover

If RNA Binding Beads are carried over into the eluate containing the RNA, they will cause the solution to be light brown in color. A small quantity of beads in the sample does not affect downstream applications such as RT-PCR or RNA amplification.

- See section "RNA binding beads were unintentionally lost" on page 21 for suggestions for avoiding bead carryover.
- To remove residual RNA Binding Beads from the purified RNA samples, place the sample plate containing the purified RNA onto the magnetic stand and recapture the RNA Binding Beads for 1 min. Then transfer the RNA solution(s) to a fresh nuclease-free plate or tubes.

A Materials not provided with the kit

Required materials not provided with the kit

Lab equipment and supplies

- General laboratory equipment including vortex mixer, microcentrifuge, pipettors, and RNase-free tips
- Magnetic stand for 96-well plates: We recommend either of the Ambion™ 96-well magnetic stands (Cat. No. AM10050, Cat. No. AM10027) for their high strength magnets and quality design.
- (Optional but recommended) Multichannel pipettor
- Orbital shaker for 96-well plates such as the Barnstead™/Lab-Line Titer Plate Shaker (VWR™ Cat. No. 57019-600 or Fisher Cat. No. 14-271-9)
- If you process fewer than 96 samples at a time, you will need additional polypropylene U bottom 96-well plates and lids.

Reagents

- 100% ethanol, ACS grade or higher quality
- 100% isopropanol, ACS grade or higher quality
- 1-bromo-3-chloropropane (BCP), e.g., MRC Cat. No. BP 151

Related products

Cat. No.	Product	Description
AM9780, AM9782, AM9784	RNase*Zap*™	RNase Decontamination Solution. RNase*Zap*™ 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*™.
AM7020, AM7021	RNA*later*™+ICE reagent	*RNAlater* reagent is an aqueous sample collection solution that stabilizes and protects cellular RNA in intact, unfrozen tissue and cell samples. RNAlater reagent eliminates the need to immediately process samples or to freeze samples in liquid nitrogen. Samples can be submerged in RNAlater reagent for storage at RT, 4°C, or –20°C without jeopardizing the quality or quantity of RNA that can be obtained.
—	RNase-free Tubes & Tips	RNase-free tubes and tips are available in most commonly used sizes and styles. They are guaranteed RNase- and DNase-free.

Cat. No.	Product	Description
AM10050	96-well Magnetic-Ring Stand	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.
AM10027	Magnetic Stand-96	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 (Part no. AM10050).
—	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 Cy™5. 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.
AM1745	MessageSensor™ RT Kit	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

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. 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)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

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  - 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

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