invitrogen

MagMAX™-96 for Microarrays Total RNA Isolation Kit

USER GUIDE

Catalog Number AM1839

Publication Number 1839M

Revision K

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**.

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

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No. 1839M

Revision	Date	Description
K	24 May 2022	The manual was updated to include automated procedures for isolation of RNA using the KingFisher™ Flex or KingFisher™ Duo Prime instrument.
J	17 December 2021	• The catalog number for the MagMAX™-96 for Microarrays Total RNA Isolation Kit was updated. • Other minor wording and formatting updates were included for style and clarity.
H	12 March 2018	Rebranded and streamlined the protocol.
G	November 2011	Revised the baseline.

Contents

1 Product information

Product description

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. The kit contains sufficient reagents to process up to 96 samples at the same time. 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. The combination of TRI Reagent™ solution and MagMAX™ technology provides a streamlined RNA purification workflow, even in high throughput format, without sacrificing RNA quantity or quality.

Contents and storage

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

Table 1 MagMAX™-96 for Microarrays Total RNA Isolation Kit (Cat. No. AM1839)

Component	Amount	Storage
Processing Plate & Lid	1 each	Room temperature
Lysis/Binding Solution Concentrate Add 6 mL 100% isopropanol before use.	12 mL	Room temperature
Wash Solution 1 Concentrate Add 6 mL 100% isopropanol before use.	18 mL	Room temperature
Wash Solution 2 Concentrate Add 58 mL 100% ethanol before use.	72.5 mL	Room temperature
Elution Buffer	14 mL	Room temperature
MagMAX™ TURBO™ DNase Buffer	6 mL	4°C or room temperature

Table 1 MagMAX-96 for Microarrays Total RNA Isolation Kit (Cat. No. AM1839) *(continued)*

Component	Amount	Storage
TRI Reagent™ solution (four 25 mL bottles)	100 mL	4°C
RNA Binding Beads	1.1 mL	4°C. Do not freeze.
Lysis/Binding Enhancer	1.1 mL	–20°C
TURBO™ DNase (10 U/µL)	250 µL	–20°C

Required materials not supplied for manual procedures

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Table 2 Equipment

Item	Source
Ambion™ 96-well Magnetic-Ring Stand	AM10050
Ambion™ Magnetic Stand-96	AM10027
Orbital shaker for 96-well plates such as the Barnstead™/Lab-Line Titer Plate Shaker	VWR™ 57019-600
Thermo Scientific™ Compact Digital Microplate Shaker	Fisher Scientific 11-676-337
Fisher Scientific™ Analog Vortex Mixer	Fisher Scientific 02-215-365
PRO250 Homogenizer with 7 × 95 mm Saw Tooth Bottom Generator Probe	PRO Scientific 01-01250 and 02-07095
Incubator capable of reaching 65°C with slatted shelves	**MLS**
Adjustable micropipettors	**MLS**
Multi-channel micropipettors	**MLS**

Table 3 Plates and combs

Item	Source
KingFisher™ 96 KF microplate (standard well plate)	97002540

Table 4 Other consumables

Item	Source
MicroAmp™ Clear Adhesive Film	4306311
Nonstick, RNase-free Microfuge Tubes, 1.5 mL	AM12450
Nonstick, RNase-Free Microfuge Tubes, 2.0 mL	AM12475
5–mL culture tubes	**MLS**
Conical Tubes (15 mL)	AM12500
RNase-free Tips (200 µL size)	AM12650
RNase-free Tips (1000 µL size)	AM12660
Aerosol-resistant pipette tips	**MLS**
Reagent reservoirs	**MLS**

Table 5 Reagents

Item	Source
Isopropanol, 100% (ACS grade or higher)	**MLS**
Ethanol, 100% (ACS grade or higher)	**MLS**
1-bromo-3-chloropropane (BCP)	MRC BP 151
2-Mercaptoethanol	**MLS**
(Optional) Chloroform	**MLS**

Required materials not supplied for automated procedures

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Table 6 Instrument

Item	Source
KingFisher™ Flex Magentic Particle Processor with 96 Deep-Well Head	5400630
KingFisher™ Duo Prime Purification System	5400110

Table 7 Equipment

Item	Source
Thermo Scientific™ Compact Digital Microplate Shaker	Fisher Scientific 11-676-337
Thermo Scientific™ Analog Vortex Mixer	Fisher Scientific 02-215-365
Incubator capable of reaching 65°C with slatted shelves	**MLS**
Adjustable micropipettors	**MLS**
Multi-channel micropipettors	**MLS**

Table 8 Plates and combs

Item[1]	Source
Deep-Well Plates, one of the following:	
KingFisher™ Flex Microtiter Deep-Well 96 plate, sterile	95040460
KingFisher™ 96 Deep-Well Plate, v-bottom, polypropylene	95040450
Standard Well Plate:	
KingFisher™ 96 KF microplate	97002540
One of the following tip combs, depending on the instrument used:	
KingFisher™ 96 tip comb for deep-well magnets	97002534
KingFisher™ 12-tip comb, for 96 deep-well plate[2]	97003500

[1] KingFisher™ Duo Combi Pack (Cat. no. 97003530) includes plates and combs for the KingFisher™ Duo Prime Magnetic Particle Processor

[2] For use with the KingFisher™ Duo Prime instrument only.

Table 9 Other consumables

Item	Source
MicroAmp™ Clear Adhesive Film	4306311
Nonstick, RNase-free Microfuge Tubes, 1.5 mL	AM12450
Nonstick, RNase-Free Microfuge Tubes, 2.0 mL	AM12475
Conical Tubes (15 mL)	AM12500
Aerosol-resistant pipette tips	**MLS**
Reagent reservoirs	**MLS**

Table 10 Reagents

Item	Source
Isopropanol, 100% (ACS grade or higher)	MLS
Ethanol, 100% (ACS grade or higher)	MLS
2-Mercaptoethanol	MLS

2 Methods

Procedural guidelines

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.

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.

Prepare reagents

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

1. 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.
2. 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.
3. 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.
4. Mark the labels of the solutions to indicate that the isopropanol or ethanol was added. Store the solutions at room temperature.

3 Sample Homogenization

Homogenize the samples

1. Homogenize samples in TRI Reagent™ solution.

   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, then 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.

3. Proceed to “Procedure overview” on page 14, “Prepare the sample and processing plates” on page 23, or “Prepare the sample and processing plates” on page 25.

4 Manual RNA isolation

Procedure overview

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, RNA isolation is performed using one of the following procedures:

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

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.

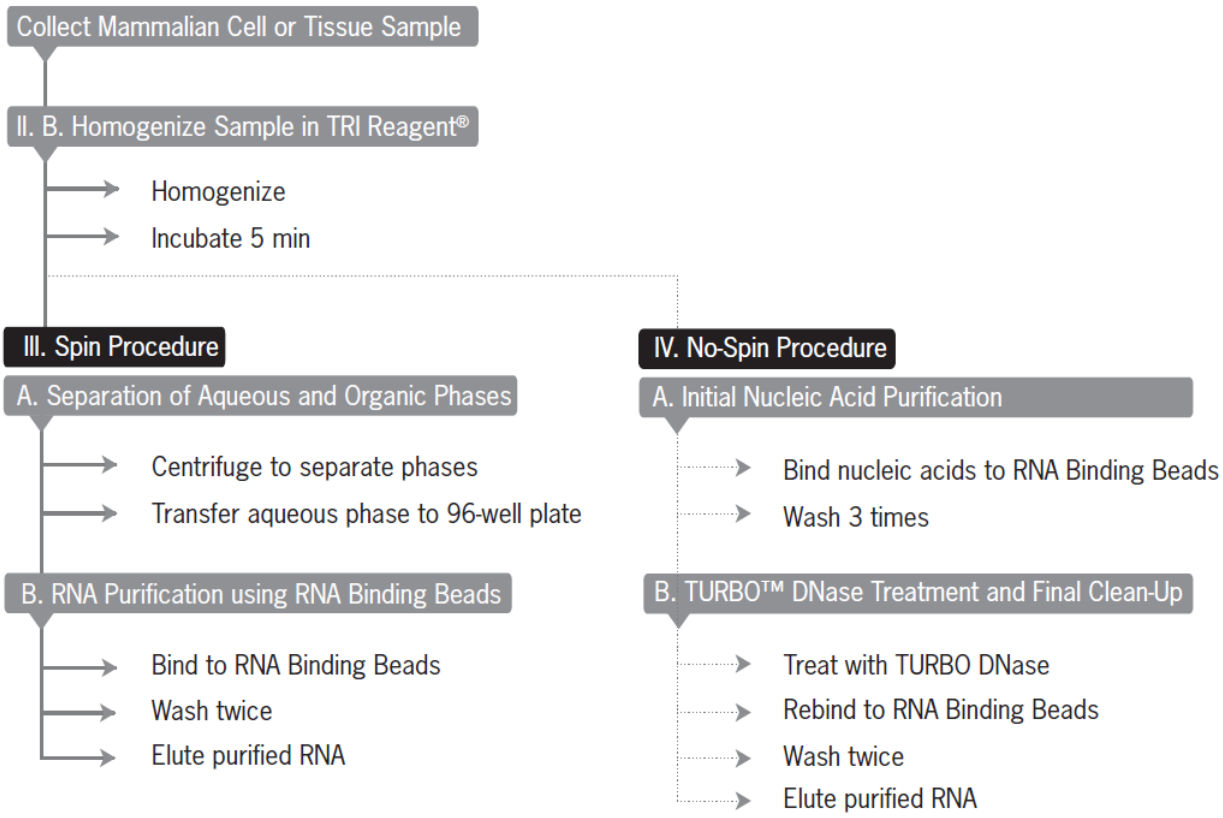
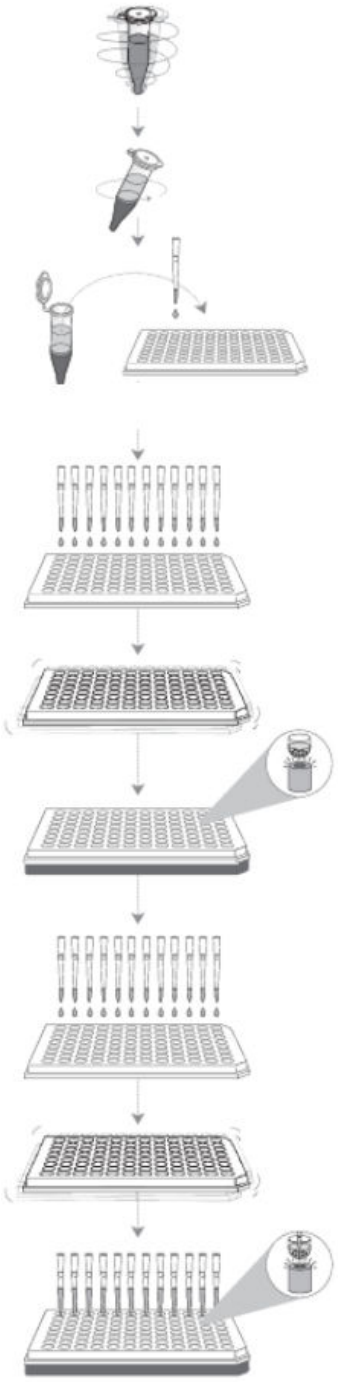


Figure 1 MagMAX™-96 for microarrays kit procedure overview

Perform the spin procedure

Workflow



Separation of Aqueous and Organic Phases

1. Mix homogenate with 0.1 volumes BCP and leave at room temp for 5 min

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

3. Transfer 100 µL aqueous phase to the Processing Plate

RNA Purification Using RNA Binding Beads

1. Add 50 µL of 100% isopropanol and shake for 1 min

2. Add 10 µL of RNA Binding Beads and shake for 3 min

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

4. Add 10 µL of RNA Binding Beads and shake for 3 min

5. Dry the beads by shaking for 2 min

6. Elute the RNA in 50 µL of Elution Buffer

Separate samples into aqueous and organic phases

1. Prepare homogenates. See “Homogenize the samples” on page 12.
2. 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), then cap the tube securely.
   b. Vortex at moderate speed for 5–10 seconds.
   c. Store the mixture at room temperature for 5 min.
3. 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).
4. 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.

Perform 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.
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.

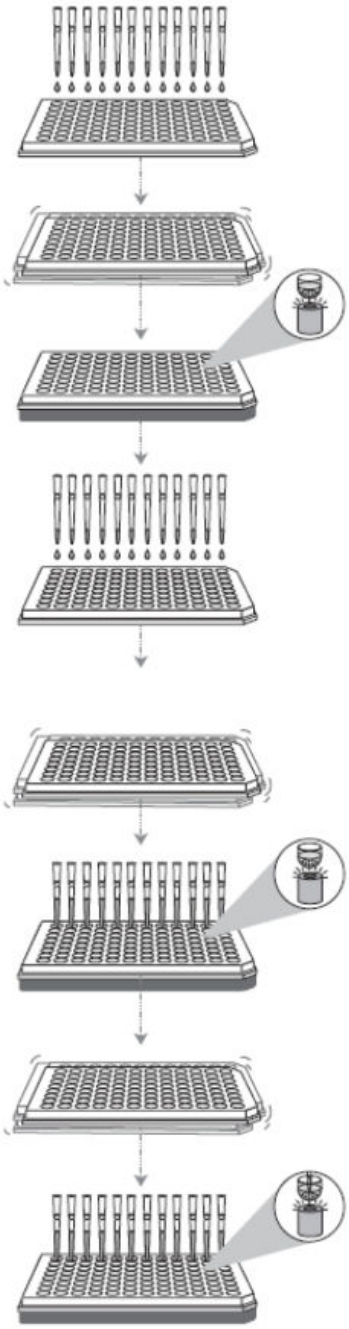
   This dries the beads, removing residual ethanol which otherwise could interfere with downstream applications.
6. Elute the RNA in 50 µL of Elution Buffer.
   a. Add 50 µL Elution Buffer to each sample and shake vigorously for 3 min at the maximum speed for lower volumes.

   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.

Perform the no-spin procedure

Workflow



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 DNase and 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

Perform initial nucleic acid purification

1. Prepare homogenates. See “Homogenize the samples” on page 12.

2. 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 3.
3. 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.
4. 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.
5. Magnetically capture the RNA Binding Beads, discard the supernatant, then 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 pipetting 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, then remove the Processing Plate from the magnetic stand.

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

6. 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, then remove the Processing Plate from the magnetic stand.

IMPORTANT! Complete removal of the supernatant is critical.

7. Wash twice with 150 µL Wash Solution 2, then 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 21.

   c. Carefully aspirate and discard the supernatant without disturbing the beads, then 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

Treat the samples with TURBO™ DNase, then perform 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.

   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.

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

3. Magnetically capture the RNA Binding Beads, then 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, then 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, then 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.
   - 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.

5 Automated RNA isolation

Download the KingFisher™ Flex or Duo program

The program required for this protocol is not pre-installed on the KingFisher™ instrument.

1. On the MagMAX™-96 Microarrays Total RNA Isolation Kit web page, scroll down to **Product Literature** section.
2. Right-click on the appropriate program for your instrument:
   - **AM1839_Spin_DW** for KingFisher™ Duo Prime
     .
   - **AM1839_Spin_DW** for KingFisher™ Flex Purification System.
3. Select **Save as Target** to download to your computer.
4. Refer to the manufacturer's documentation for instructions for installing the program on the instrument.

Isolate RNA using the KingFisher™ Flex Purification System

Prepare the sample and processing plates

This protocol describes RNA isolation using the KingFisher™ Flex Magnetic Particle Processor 24DW. If samples were frozen, thaw them completely to room temperature before proceeding.

1. Set up the processing plates. See “Set up the processing plates” on page 24.
2. Prepare homogenates. See “Homogenize the samples” on page 12.
3. Separate and remove aqueous phase from each sample. See “Separate samples into aqueous and organic phases” on page 17.
4. Transfer to 100 µL of each separated aqueous phase (fresh or thawed) to a separate well in a KingFisher™ 96 Deep-Well Plate.
5. Add 50 µL of isopropanol to each sample.
6. Add 10 µL of the RNA Binding Beads to each sample.

   Note: Vortex the RNA Binding Beads well before adding to the sample.
7. Proceed immediately to “Bind, wash, and elute the RNA” on page 24.

Set up the processing plates

While the samples are incubating, set up the Wash, DNase, Elution, and Tip Comb Plates outside the instrument as described in the following table.

Table 11 Processing plates

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Standard	Wash Solution 2	150 µL
Wash Plate 2	3	Standard	Wash Solution 2	150 µL
Elution Plate	4	Standard	Elution Buffer	50 µL
Tip Comb	5	Deep Well or standard	Place a KingFisher™ 96 tip comb for deep-well magnets in a KingFisher™ 96 Deep-Well Plate or in a KingFisher™ 96 KF microplate.	

[1] Position on the instrument

Bind, wash, and elute the RNA

1. Ensure that the instrument is set up for processing with the deep well magnetic head, then select **AM1839_Spin_DW** for KingFisher™ Flex Magnetic Particle Processor 96DW.
2. Start the run, then load the prepared processing plates in their positions when prompted by the instrument (see "Set up the processing plates" on page 24).
3. Load the sample plate (containing lysate, isopropanol, and RNA Binding Beads) at position 1 when prompted by the instrument.
4. At the end of the run (approximately 15 minutes after the initial start), remove the Elution Plate from the instrument, then seal immediately with a new MicroAmp™ Clear Adhesive Film.
   - *(Optional)* Eluates can be transferred to a storage plate after collection.
   - If excess bead residue is seen in the wells, place the Elution Plate on the Magnetic Stand-96 to capture any residue prior to downstream use of the RNA.

IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.

The purified samples are ready for immediate use. Alternatively, store the covered Elution Plate:

- On ice for up to 8 hours.
- At –20°C or –80°C for long-term storage.

Isolate RNA using the KingFisher™ Duo Prime instrument

Prepare the sample and processing plates

This protocol describes RNA isolation using the KingFisher™ Duo Prime Magnetic Particle Processor. If samples were frozen, thaw them completely to room temperature before proceeding.

1. Set up the processing plates. See “Set up the processing plate” on page 25.
2. Prepare homogenates. See “Homogenize the samples” on page 12.
3. Separate and remove aqueous phase from each sample.. See “Separate samples into aqueous and organic phases” on page 17.
4. Transfer to 100 µL of each separated aqueous phase (fresh or thawed) to a separate well of row H in the KingFisher™ 96 Deep-Well Plate processing plate prepared during step 1.
5. Add 50 µL of isopropanol to each sample.
6. Add 10 µL of the prepared RNA Binding Beads to each sample.

   Note: Vortex the RNA Binding Beads well before adding to the sample.

7. Proceed immediately to “Bind, wash, and elute the RNA” on page 26.

Set up the processing plate

Add processing reagents as indicated in the following table.

Table 12 Volume of processing reagents and plate location

Row ID	Plate row[1]	Reagent	Volume per well
Elution	A	Elution Buffer	50 µL
Tip Comb	B	Place a KingFisher™ Duo 12-Tip Comb in Row B.	
Empty	C	–	–
Empty	D	–	–
Wash 2	E	Wash Solution 2	150 µL
Wash 2	F	Wash Solution 2	150 µL
Empty	G	–	–

[1] Row on the KingFisher™ 96 Deep Well Plate.

Bind, wash, and elute the RNA

1. Ensure that the instrument is set up for processing with the deep well 96–well plates, then select the program **AM1839_Spin_DW** on the instrument.
2. Start the run, then load the prepared processing plate when prompted by the instrument.
3. At the end of the run (approximately 17 minutes after the initial start), remove the Processing plate from the instrument and transfer the eluted RNA (Row A) to an Elution Plate or individual tubes.
4. Seal plate immediately with a new MicroAmp™ Clear Adhesive Film or close tubes.

IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.

The purified samples are ready for immediate use. Alternatively, store the covered Elution Plate:

- On ice for up to 8 hours.
- At –20°C or –80°C for long-term storage.

6 Determine RNA yield and purity

Determine 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 manufacturer's instructions for using the assay.

Determine RNA quality

- Microfluidic analysis

  The Agilent™ 2100 Bioanalyzer™ instrument with Caliper™'s RNA LabChip™ Kit provides better qualitative data than conventional gel analysis for characterizing RNA. When used with the Ambion™ RNA 6000 Ladder (Cat. 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 : **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.

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.

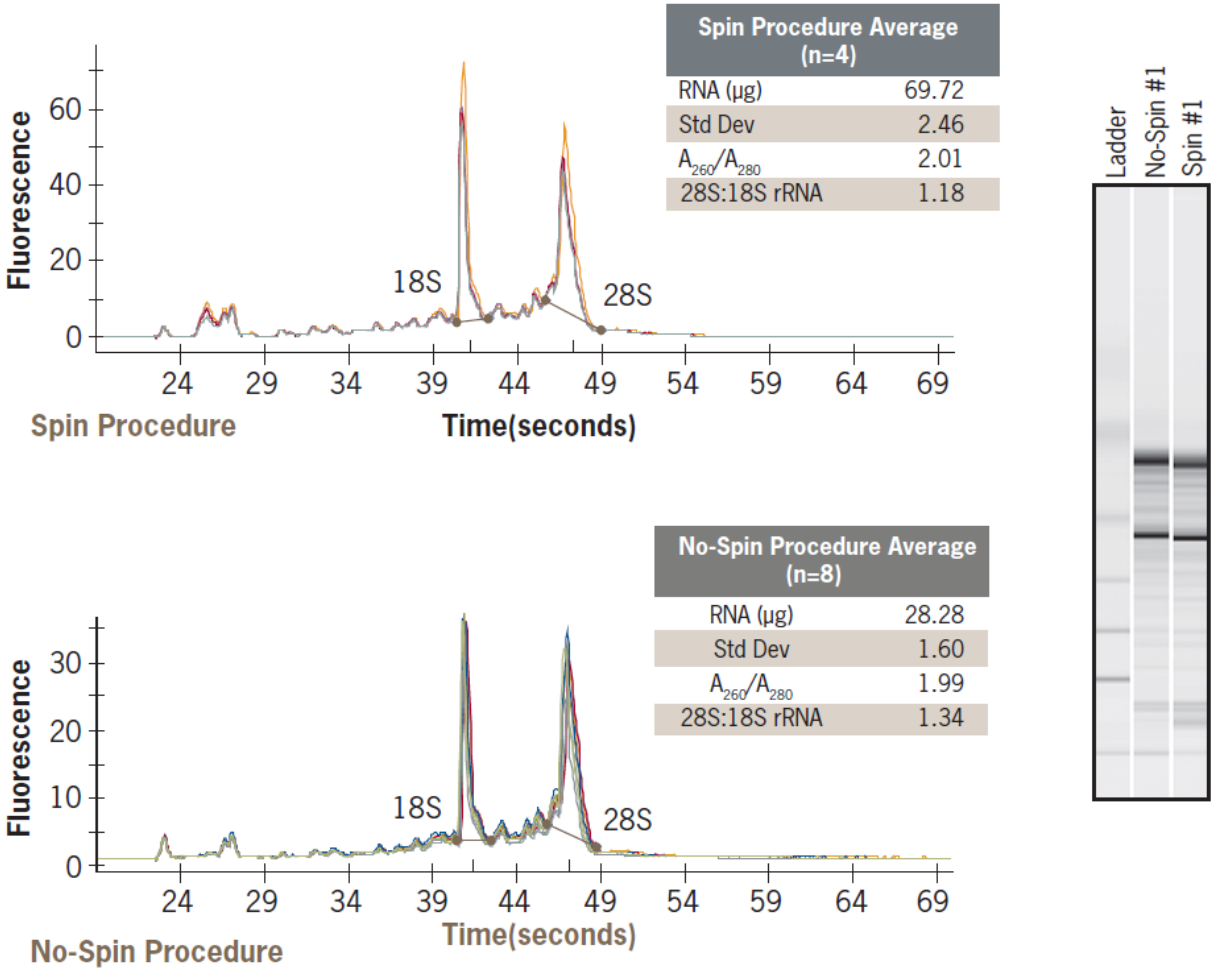


Figure 2 Consistent yield, purity, and integrity of RNA isolated with the MagMAX™-96 for microarrays kit. RNA was isolated from frozen mouse liver with the MagMAX™-96 for microarrays kit. Tissue homogenates were processed in quadruplets or octuplets using the spin procedure (homogenate derived from 10 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.

A

Troubleshooting

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 sample 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 (Cat. 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 ""Perform initial nucleic acid purification" on page 19", 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 29 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.

B

Ordering information

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Table 13 MagMAX™-96 for Microarrays Total RNA Isolation Kit (Cat. No. AM1839) contents

Item	Source
MagMAX™ Lysis/Binding Solution Concentrate	AM8500
MagMAX™ Wash Solution 1 Concentrate	AM8504
MagMAX™ Wash Solution 2 Concentrate	AM8640
MagMAX™ Total RNA Elution Buffer	A41043
TURBO™ DNase (2 U/µL)	AM2239
TRI Reagent™ Solution	AM9738
RNA Binding Beads	MLS

Table 14 Magnetic particle processor

Item	Source
MagMAX™ Express-96 Deep Well Magnetic Particle Processor	—[1]
KingFisher™ Flex Magnetic Particle Processor 96DW	5400630
KingFisher™ Apex with 96 Deep–Well head	5400930
KingFisher™ Duo Prime Magnetic Particle Processor	5400110

[1] Not available for sale.

Table 15 Plates and combs

Item[1]	Source
Deep Well Plates, one of the following:	
KingFisher™ Flex Microtiter Deep-Well 96 plate, sterile	95040460
KingFisher™ 96 Deep-Well Plate, v-bottom, polypropylene	95040450
One of the following tip combs, depending on the magnetic particle processor used:	
KingFisher™ 96 tip comb for deep-well magnets	97002534
KingFisher™ 12-tip comb, for 96 deep-well plate[2]	97003500

[1] KingFisher™ Duo Combi Pack (Cat. no. 97003530) includes plates and combs for the KingFisher™ Duo Prime Magnetic Particle Processor.

[2] For use with the KingFisher™ Duo Prime instrument only.

Table 16 Other related products

Item	Source
TURBO DNA-*free*™ Kit	AM1907
RNase*Zap*™	AM9780, AM9782, or AM9784
RNA*later*™+ICE reagent	AM7020 or AM7021
Ambion™ RNA 6000 Ladder	AM7152
MessageAmp™ II aRNA Amplification Kits	AMB17515 or AM1751
SuperScript™ IV First-Strand Synthesis System	18091050 or 18091200

C

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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

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. 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
  https://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

D 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/or its affiliate(s) 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 any questions, please contact Life Technologies at **www.thermofisher.com/support**.