PureLink™ Micro-to-Midi Total RNA Purification System

For scalable purification of total RNA from a large variety of samples

Catalog no. 12183-018

Version D
15 March 2006
25-0452

User Manual

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Kit Contents and Storage

Shipping and Storage

All components of the PureLink™ Micro-to-Midi Total RNA Purification System are shipped at room temperature. Upon receipt, store all components at room temperature.

Contents

The components included in the PureLink™ Micro-to-Midi Total RNA Purification System are listed below. Sufficient reagents are included in the kit to perform 50 isolations.

Component	Amount
RNA Lysis Solution	125 ml
Wash Buffer I	50 ml
Wash Buffer II	15 ml
RNase-Free Water	15 ml
RNA Spin Cartridges with collection tubes	50
RNA Wash Tubes	50
RNA Recovery Tubes	50

Product Qualification

The PureLink™ Micro-to-Midi Total RNA Purification System is functionally qualified as described below:

Total RNA is purified from 5×10^7 mammalian cells using the protocols and reagents described in this manual. Agarose gel electrophoresis must show two distinct ribosomal RNA bands and an mRNA smear, and must show that the RNA is intact. UV absorbance at 260/280 nm is measured to determine the yield and purity of RNA.

In addition, each kit component must be free of ribonuclease contamination and is lot qualified for optimal performance.

Accessory Products

Additional Products

The following related products are available separately from Invitrogen. For more details about these or other products, visit our website at www.invitrogen.com or contact Technical Service (page 42).

Product	Quantity	Catalog no.
RT-PCR and qRT-PCR		
SuperScript™ III First-Strand Synthesis System for RT-PCR	50 reactions	18080-051
SuperScript™ III First-Strand Synthesis SuperMix	50 reactions	18080-400
SuperScript™ III First-Strand Synthesis SuperMix for qRT-PCR	50 reactions 250 reactions	11752-050 11752-250
Platinum® PCR SuperMix	100 reactions	11306-016
Platinum® Quantitative PCR SuperMix-UDG	100 reactions 500 reactions	11730-017 11730-025
SuperScript™ III Two-Step qRT-PCR Kit	100 reactions	111734-050
SuperScript™ III Two-Step qRT-PCR Kit with ROX	100 reactions	11146-100
LUX™ Fluorogenic Primer Sets	50 nmol or 200 nmol	Design and order at www.invitrogen.com/lux
Other Products		
Homogenizer	50 pack	12183-026
RNase *AWAY*®	250 ml	10328-011
TRIzol® Reagent	100 ml	15596-026
TRIzol® LS Reagent	100 ml	10296-101
TRIzol® Max™ Bacterial RNA Isolation Kit	100 reactions	16096-020
PureLink™ Plant RNA Reagent	100 ml	12322-012
DNase I, Amplification Grade (1 unit/ µl)	100 units	18068-015
0.16–1.77 Kb RNA Ladder	75 µg	15623-010
UltraPure™ DEPC-treated Water	1 L	750023
UltraPure™ DNase/RNase-Free Distilled Water	500 ml	10977-015
Quant-iT™ RNA Assay Kit	1 kit	Q33140

Introduction

Overview

Introduction

The PureLink™ Micro-to-Midi Total RNA Purification System provides a simple, reliable, and rapid method for isolating high-quality total RNA from a wide variety of samples, including animal and plant cells and tissue, blood, bacteria, yeast, and liquid samples (*e.g.*, cytoplasmic RNA from fresh animal cells, RNA from various enzymatic reactions, RNA clean-up samples). The purified total RNA is suitable for use in any downstream application.

System Overview

You can use the sample-specific protocols in this manual to isolate total RNA from a wide range of sample types and amounts. In general, samples are lysed and homogenized in the presence of guanidinium isothiocyanate, a chaotropic salt capable of protecting the RNA from endogenous RNases (Chirgwin *et al.*, 1979). After homogenization, ethanol is added to the sample, and then the sample is processed through a spin cartridge.

RNA binds to the silica-based membrane in the spin cartridge, and impurities are effectively removed by washing (Vogelstein & Gillespie, 1979). The purified total RNA is eluted in water and is suitable for use in a variety of applications, including RT-PCR, real-time quantitative RT-PCR (qRT-PCR), Northern blotting, nuclease protection assays, RNA amplification for microarray analysis, and cDNA library preparation after poly(A)+ selection.

Advantages of the System

The PureLink™ Micro-to-Midi Total RNA Purification System offers the following advantages:

- RNA isolation from a wide variety of sample types and amounts
- Large scalability from micro to midi
- Minimal genomic DNA contamination of the purified RNA and optional on-column DNase digestion
- Rapid and convenient column purification procedures
- Reliable performance of high-quality purified total RNA in downstream applications

System Specifications

Starting Material:	Varies
Column Binding Capacity:	~1 mg nucleic acid
Column Reservoir Capacity:	700 µl
Wash Tube Capacity:	2.0 ml
Centrifuge Compatibility:	Capable of centrifuging >12,000 × *g*
Elution Volume:	30–100 µl
RNA Yield:	Varies with sample type and quality

Continued on next page

Overview, continued

Starting Material

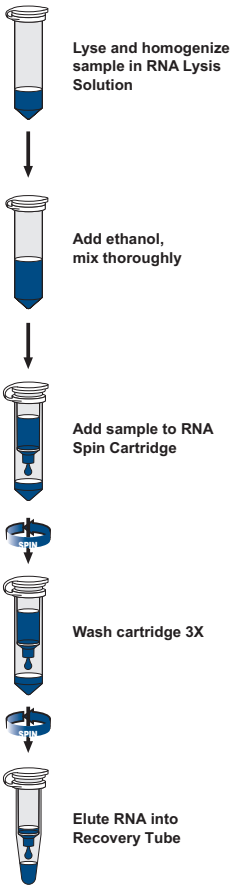
The various sample types and amounts that can be processed using the system are listed in the table below:

Sample type	Amount
Animal and plant cells	≤ 1×10^8 cells
Animal tissue	≤ 200 mg
Plant tissue	≤ 250 mg
Whole blood	≤ 0.2 ml
Yeast cells	≤ 5×10^8 cells
Bacterial cells	≤ 1×10^9 cells
Liquid samples*	≤ 1.2 ml

*Including cytoplasmic RNA extracts from mammalian cells, *in vitro* transcription reactions, DNase I digestions, RNA labeling reactions, and RNA clean-up preps.

Workflow

The flow chart illustrates the steps for isolating total RNA using the PureLink™ Micro-to-Midi Total RNA Purification System:



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Overview, continued

Sample Lysis and Homogenization

Use the following table to determine the best method for lysing and homogenizing your sample type. Refer to the following pages for further details on methods of sample lysis and homogenization.

Sample Type	Lysis Options	Homogenization Options	Remarks
Animal and Plant Cells	RNA Lysis Solution, vortexing	• Homogenizer • Syringe and needle • Rotor-stator	Rotor-stator is required for homogenization of $>10^7$ cells.
Animal Tissue: Frozen or Fresh Fibrous	Pestle with microcentrifuge tube (≤10 mg tissue)	• Homogenizer • Syringe and needle	
	Mortar and pestle in liquid nitrogen (10–100 mg tissue)	• Homogenizer • Syringe and needle	
	Rotor-stator (≤200 mg tissue)		Rotor-stator lyses and homogenizes simultaneously, and can be used with all tissue amounts up to 200 mg.
Animal Tissue: Fresh Soft	Pestle with microcentrifuge tube (≤100 mg tissue)	• Homogenizer • Syringe and needle	
	Rotor-stator (≤200 mg tissue)		Rotor-stator lyses and homogenizes simultaneously, and can be used with all tissue amounts up to 200 mg.
Plant Tissue: Frozen or Fresh Fibrous	Mortar and pestle in liquid nitrogen	• Homogenizer • Rotor-stator	We recommend using a mortar and pestle with liquid nitrogen for more complete lysis than rotor-stator alone
Plant Tissue: Fresh Soft	Rotor-stator		Rotor-stator lyses and homogenizes simultaneously
Fresh Whole Blood	RNA Lysis Solution, vortexing		
Yeast Cells	Enzyme digestion by zymolase followed by RNA Lysis Solution, vortexing		Not recommended for kinetic experiments.
	Mortar and pestle with crushed dry ice	• Homogenizer • Syringe and needle • Rotor-stator	Recommended for kinetic experiments.
Bacteria	Digestion with lysozyme, vortexing	• Homogenizer • Syringe and needle • Rotor-stator	
Liquid samples	RNA Lysis Solution, vortexing		

Methods

General Information

Introduction

Review the information in this section before starting. Guidelines are provided for handling RNA, sample collection, handling system reagents, and methods for lysis and homogenization.

Guidelines for Handling RNA

Follow the guidelines below to prevent RNase contamination and to maximize the RNA yield:

- Use disposable, individually wrapped, sterile plastic ware
- Use only sterile, disposable RNase-free pipette tips and microcentrifuge tubes
- Wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin; change gloved frequently, particularly as the protocol progresses from crude extracts to more purified material (*e.g.,* from Wash Buffer I to Wash Buffer II).
- Always use proper microbiological aseptic techniques when working with RNA
- Use RNase *AWAY*® Reagent (for catalog number, see page vi) to remove RNase contamination from work surfaces and non-disposable items such as centrifuges and pipettes used during purification.

Guidelines for Sample Collection

Use the following guidelines for collecting your samples to minimize RNA degradation prior to RNA purification and to maximize the RNA yield:

- Always wear disposable gloves while handling samples and reagents to prevent RNase contamination.
- Work quickly during sample harvesting, use RNase-free dissection tools and containers (scalpels, dishes, tubes etc.) and work on RNase-free work surfaces (use RNase *AWAY*® Reagent).
- To purify total RNA from fresh samples, keep fresh cell and tissue samples on ice immediately after harvesting; quickly proceed to adding RNA Lysis solution, sample lysis and homogenizaton.
- To purify total RNA from frozen samples, freeze samples immediately after harvesting in liquid nitrogen or on dry ice. Keep frozen samples at -80° or in liquid nitrogen until proceeding to sample lysis and homogenization.
- **Whole blood:** We recommend collecting whole blood in the presence of anticoagulants such as EDTA or citrate and store at 4ºC until use. Freshly drawn blood can be used without anticoagulants. You may also process frozen blood.

Continued on next page

Some of the PureLink™ Micro-to-Midi Total RNA Purification System buffers contain hazardous chemicals.

- Both the RNA Lysis Solution and Wash Buffer I contain guanidine isothiocyanate. This chemical is harmful if it comes in contact with the skin, inhaled, or swallowed. Always wear a laboratory coat, disposable gloves, and goggles when handling solutions containing this chemical.
- Do not add bleach or acidic solutions directly to solutions containing guanidine isothiocyanate or sample preparation waste. Guanidine isothiocyanate forms reactive compounds and toxic gases when mixed with bleach or acids.
- Solutions containing ethanol are considered flammable. Use appropriate precautions when using this chemical.

Always wear a laboratory coat, disposable gloves, and eye protection when handling buffers. Dispose of the buffers in appropriate waste containers.

DNase I Treatment of RNA

If your downstream application requires DNA-free total RNA (*e.g.,* microarray analysis or qRT-PCR for low-copy target sequences), you can use a convenient on-column DNase I treatment during the purification procedure. Each sample-specific protocol refers you to the on-column DNase I treatment (page 38) at the appropriate point in the procedure. The on-column DNase I treatment reduces the loss of RNA compared to DNase I treatment of the RNA after purification (page 39).

Microcentrifuge Pestle

RNase-free microcentrifuge pestles allow disruption and lysis of tissue samples in a microcentrifuge tube. They are usually made of teflon, polyethylene, or stainless steel, and are designed to fit standard microcentrifuge tube sizes (*e.g.,* conical 1.5-ml tubes or 2-ml round-bottom tubes).

To use the pestle, transfer the tissue sample into the microcentrifuge tube cooled on ice, add RNA Lysis Solution, and use up-and-down and twisting movements to disrupt the sample between the tube wall and the pestle. After lysis, homogenize the sample as specified in the sample-specific protocols.

Mortar and Pestle

RNase-free mortars and pestles are used to disrupt and lyse tissue. They are used in combination with liquid nitrogen for frozen and fibrous tissue samples. Place your sample and a small amount of liquid nitrogen into the mortar and grind into powder using the pestle. Transfer the frozen tissue powder into a liquid nitrogen-cooled tube of appropriate size and allow the liquid nitrogen to evaporate. Ensure that the sample does not thaw before you add the appropriate amount of RNA Lysis Solution to the sample, as directed in the protocols.

After lysis, homogenize the sample as specified in the sample-specific protocols.

Continued on next page

Homogenizer

The Homogenizer from Invitrogen is designed to homogenize cell or tissue lysates via centrifugation prior to nucleic acid purification. See page vi for ordering information. The Homogenizer consists of a cartridge with a specialized membrane that fits inside a collection tube containing the lysate. The tube is placed in a microcentrifuge, and the Homogenizer homogenizes the lysate by centrifugal force (12,000 × *g* for 2 minutes).

The Homogenizer provides highly consistent results and is more convenient than other homogenization methods. It is especially effective for clarifying particulates from plant tissues. For more details, visit our web site at www.invitrogen.com or contact Technical Support (page 42).

Rotor-Stator Homogenizer

Rotor-stator homogenizers allow simultaneous lysis and homogenization of tissue samples or cell lysates by the shearing force of a fast rotating probe.

To use a rotor-stator, you transfer your sample into a round-bottomed tube of appropriate size and add the appropriate volume of RNA Lysis Buffer. Then you insert the rotor-stator probe tip into the sample and homogenize for 5–90 seconds, depending on the toughness of sample. Avoid foaming by keeping the tip of the probe submerged in the lysis solution while holding the tip against the tube wall. See the documentation provided with your rotor-stator for more information.

Rotor-stators are available in various sizes. Common models include the hand-held ULTRA-TURRAX® (IKA Works, Inc.) and Polytron® Homogenizer (Kinematica, sold by Brinkmann Instruments, Westbury, NY).

Buffer Preparation and Parameters

Preparing Wash Buffer II with Ethanol

Before using the Wash Buffer II for the first time, add 60 ml of 96–100% ethanol directly to the bottle. Check the box on the Wash Buffer II label to indicate that ethanol was added.

Preparing RNA Lysis Solution with 2-Mercaptoethanol

Prepare the amount of RNA Lysis Solution needed for each purification procedure fresh for each use by adding 1% (v/v) 2-mercaptoethanol. Add 10 µl of 2-mercaptoethanol to each 1 ml of RNA Lysis Solution. Use the tables provided with each sample-specific protocol to determine the volume of RNA Lysis Solution with 2-mercaptoethanol needed.

Amount of RNA Lysis Solution

The amount of RNA Lysis Solution is determined by the amount of starting material. Each sample-specific protocol includes the recommended amount of RNA Lysis Solution for the specified sample types and amounts. If your sample contains more than an average amount of RNA, use less starting material or increase the amount of RNA Lysis Solution. Be careful to not exceed the maximum binding capacity of the column (~1 mg nucleic acid) when selecting the amount of starting material, as this will decrease the total RNA yield.

Elution Parameters

Elution Buffer

The RNA can be eluted from the RNA Spin Cartridge using RNase-Free water (included in the kit). Alternatively, you may use Tris Buffer (10 mM Tris-HCl), pH 7.5 in RNase-free water.

Elution Buffer Volume

Use between 30–100 µl of Elution Buffer for each elution, depending on sample source, amount of starting material, and desired RNA concentration. RNA yield is dependent on tissue type, size, and quality of the specimen. Example yields for various sample types and amounts are provided on page 32.

General Recommendations for Elution Buffer Volume

For the **highest concentration** of RNA, use a smaller volume of Elution Buffer. For best results, always use at least 30 µl. Note that a small elution volume may result in a reduced total yield of RNA.

For the **highest yield** of RNA, use up to 100 µl of Elution Buffer. You may perform sequential elutions of 30 µl each, collected into the same tube, which can lead to an increased yield. Note that increasing the elution volume will decrease the concentration of purified RNA.

Note

In previous versions of this manual, specific elution volumes were recommended based on the expected yield of RNA. Since the elution volume can vary depending on whether you want a higher concentration or a higher yield of purified RNA, we have changed our recommendations to the less rigid guidelines specified above. If you are an experienced user of this system, you can continue using the elution volumes as previously specified or you can follow the new guidelines in this manual.

Purifying RNA from Animal and Plant Cells

Introduction

This section provides instructions for preparing total RNA from animal and plant cells. Separate protocols are provided for ≤1 × 10^7 cells (monolayer or suspension) and 1 × 10^7–1 × 10^8 cells.

Note

For samples that are difficult to lyse, you can use TRIzol® Reagent followed by purification using the PureLink™ Micro-to-Midi Total RNA System. See the protocol on page 36.

Materials Needed

You will need the following items in addition to the components provided in the kit:

- 2-mercaptoethanol
- 70% ethanol (in RNase-free water)
- Microcentrifuge capable of centrifuging 12,000 × *g*
- 1.5-ml RNase-free microcentrifuge tubes
- RNase-free tubes, 15 ml (>10^7 cells per sample)
- PBS (>10^7 cells per sample)
- RNase-free pipette tips
- **For ≤1 × 10^7 cells:**

  Homogenizer (see page 6) ***or*** RNase-free syringe (1 ml) with 18-21 gauge needle ***or*** rotor-stator homogenizer (see page 6)
- **For 1 × 10^7–1 × 10^8 cells:**

  Rotor-stator homogenizer (see page 6)

Preparing RNA Lysis Solution

Prepare the RNA Lysis Solution fresh for each use by adding 1% (v/v) 2-mercaptoethanol (*e.g.,* add 10 µl of 2-mercaptoethanol to every 1 ml of RNA Lysis Solution). Use the table below to determine the amount of RNA Lysis Solution needed per sample.

Number of cells	RNA Lysis Solution (ml)
≤5 × 10^6	0.3*
5 × 10^6–5 × 10^7	0.6
5 × 10^7–5 × 10^8	1.2

* Use 0.5 ml if using rotor-stator for lysis or homogenization

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Lysis and Homogenization: ≤1 × 10^7 Suspension Cells

Follow the steps below to prepare lysates from ≤1 × 10^7 suspension cells:

1. Transfer cells to an appropriate tube and centrifuge at 2,000 × *g* for 5 minutes at 4°C to pellet.
2. Remove the growth medium from the tube, and add the volume of RNA Lysis Solution specified in the table on page 8.
3. Vortex at high speed until the cell pellet is completely dispersed and the cells appear lysed. (**Note:** If you are using a rotor-stator homogenizer, you may skip this step.)
4. Proceed with **one** of the following homogenization options at room temperature:
   - Transfer lysate to the Homogenizer inserted in a collection tube and centrifuge at 12,000 × *g* for 2 minutes. Remove the Homogenizer cartridge when done.
   - Pass the lysate 5–10 times through an 18–21-gauge needle attached to an RNase-free syringe.
   - Transfer the lysate to an appropriately sized tube and homogenize using a rotor-stator homogenizer at maximum speed for at least 45 seconds. Centrifuge the homogenate at ~2,600 × *g* for 5 minutes, and then transfer supernatant to a clean tube.

Proceed to **Binding, Washing, and Elution**, page 11.

Lysis and Homogenization: ≤1 × 10^7 Monolayer Cells

Follow the steps below to prepare lysates from ≤1 × 10^7 monolayer cells:

1. Remove the growth medium from the cells.
2. Pipet the volume of RNA Lysis Solution specified in the table on page 8 evenly over the surface of the monolayer, and pipet up and down until the cells appear lysed.
3. Proceed with **one** of the following homogenization options at room temperature:
   - Transfer the lysate to the Homogenizer inserted in a collection tube and centrifuge at 12,000 × *g* for 2 minutes. Remove the Homogenizer cartridge from the tube when done.
   - Transfer the lysate to a 1.5-ml tube and pass 5–10 times through an 18–21-gauge needle attached to an RNase-free syringe.
   - Transfer the lysate to an appropriately sized tube and homogenize using a rotor-stator homogenizer at maximum speed for at least 45 seconds. Centrifuge the homogenate at ~2,600 × *g* for 5 minutes, and then transfer supernatant to a clean tube.

Proceed to **Binding, Washing, and Elution**, page 11.

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Purifying RNA from Animal and Plant Cells, continued

Lysis and Homogenization: 1×10^7–1×10^8 Monolayer or Suspension Cells

Follow the steps below to prepare lysates from 1×10^7–1×10^8 cells. This protocol uses a rotor-stator homogenizer. Use 15-ml tubes to compensate for volume expansion during homogenization using a rotor-stator.

1. **Monolayer cells:** Trypsinize cells and transfer detached cells to an appropriate tube. Centrifuge at 2,000 × *g* for 5 minutes at 4°C to pellet.

   Suspension cells: Transfer cells to an appropriate tube and centrifuge at 2,000 × *g* for 5 minutes at 4°C to pellet.
2. Remove the supernatant and add the volume of RNA Lysis Solution specified in the table on page 8 to the pellet.
3. Vortex at high speed until the cell pellet is completely dispersed and the cells appear lysed.
4. Homogenize cells using a rotor-stator homogenizer at maximum speed for at least 45 seconds.
5. Centrifuge the homogenate at ~2,600 × *g* for 5 minutes at room temperature.
6. Transfer the supernatant to a clean 15-ml tube.

Proceed to **Binding, Washing, and Elution**, next page.

Lysis and Homogenization: Frozen Cell Pellets

Follow the steps below to prepare lysates from frozen cell pellets. For 1×10^7–1×10^8 cells, we recommend homogenizing with a rotor-stator homogenizer.

1. In an appropriate tube, add the volume of RNA Lysis Solution specified in the table on page 8 to the cell pellet.
2. Vortex at high speed until the cell pellet is completely dispersed and the cells appear lysed. (**Note:** If you are using a rotor-stator homogenizer, you may skip this step.)
3. Proceed with **one** of the following homogenization options at room temperature:
   - Transfer lysate to the Homogenizer inserted in an RNase-free tube and centrifuge at 12,000 × *g* for 2 minutes. Remove the Homogenizer cartridge when done.
   - Pass the lysate 5–10 times through an 18–21-gauge needle attached to an RNase-free syringe.
   - Transfer the lysate to an appropriate tube and homogenize using a rotor-stator homogenizer at maximum speed for at least 45 seconds. Centrifuge the homogenate at ~2,600 × *g* for 5 minutes, and then transfer supernatant to a clean tube.

Proceed to **Binding, Washing, and Elution**, next page.

Continued on next page

Binding, Washing, and Elution

Follow the steps below to bind, wash, and elute your sample:

1. Add one volume of 70% ethanol to each volume of cell homogenate (prepared as described on pages 9–10). **Note:** If part of the sample was lost during homogenization, adjust the volume of ethanol accordingly.
2. Mix thoroughly by vortexing. Disperse any visible precipitate that may form after adding ethanol.
3. Transfer up to 700 µl of the sample (including any remaining precipitate) to the RNA Spin Cartridge pre-inserted in a collection tube. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.

   Note: If you are processing the maximum starting amount of sample you may centrifuge for up to 10 minutes to completely pass the lysate through the RNA Spin Cartridge.
4. Repeat Step 3 until the entire sample has been processed.

   ***Optional*:** If your downstream application requires DNA-free total RNA, you may use the convenient on-column DNase I treatment at this point in the procedure (see page 38).
5. Add 700 µl of Wash Buffer I to the spin cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through and the collection tube.
6. Place the spin cartridge into a clean RNA Wash Tube, provided in the kit.
7. Add 500 µl Wash Buffer II with ethanol (prepared as described on page 7) to the spin cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.
8. Repeat Step 7 once.
9. Centrifuge the spin cartridge at 12,000 × *g* for 1 minute to dry the membrane with attached RNA. Discard the collection tube, and insert the cartridge into an RNA Recovery Tube supplied with the kit.
10. To elute the RNA, add 30–100 µl of RNase-free water to the center of the spin cartridge, and incubate at room temperature for 1 minute. See page 7 for detailed elution parameters.
11. Centrifuge the spin cartridge for 2 minutes at ≥12,000 × *g* at room temperature. If you are performing sequential elutions, collect all elutes into the same tube.

To determine the quantity and quality of the RNA, see page 30. To perform DNase I digestion of the purified RNA, see page 39. If you will use the RNA within a few hours, store on ice. For longer storage, store at –80°C.

Purifying RNA from Animal Tissue

Introduction

This section provides protocols for purifying total RNA from up to 200 mg of fresh or frozen animal tissue.

Note

- Protocols for frozen tissue/fibrous fresh tissue are provided starting on the next page. Protocols for soft fresh tissue are provided starting on page 15.
- For samples that are difficult to lyse, you can use TRIzol® Reagent followed by purification using the PureLink™ Micro-to-Midi Total RNA System. See the protocol on page 36.

Important

- Frozen tissue must remain frozen at –80°C prior to lysis. Cool tubes in dry ice before placing frozen tissue in them. Thawing of frozen tissue prior to lysis may result in RNA degradation and loss of RNA yield.
- Fast and complete disruption of tissue during the lysis step is important to prevent RNA degradation.

Additional Materials Needed

You will need the following additional items not provided in the kit:

- 2-mercaptoethanol
- 70% ethanol (in RNase-free water)
- 96–100% ethanol
- One of the following for tissue disruption and lysis: Glass, teflon, or plastic pestle (for ≤100 mg tissue) ***or*** rotor-stator homogenizer (required for ≥100 mg of tissue; see page 6)
- One of the following for homogenization: 18–20-gauge needle with RNase-free syringe ***or*** Homogenizer (see page 6) ***or*** rotor-stator homogenizer
- RNase-free tubes (1.5 ml–15 ml, depending on amount of tissue)
- Liquid nitrogen (for lysis of ≥10 mg of frozen tissue or fresh fibrous tissue)
- Microcentrifuge capable of centrifuging 12,000 × *g*
- RNase-free pipette tips

Preparing RNA Lysis Solution

Prepare the RNA Lysis Solution **fresh** for each use by adding 1% (v/v) 2-mercaptoethanol (*e.g.*, add 10 µl of 2-mercaptoethanol to every 1 ml of RNA Lysis Solution). Use the table below to determine the amount of RNA Lysis Solution needed per sample.

Amount of tissue	RNA Lysis Solution (ml)
≤10 mg	0.3*
10–60 mg	0.6
60–100 mg	1.2
100–200 mg	2.4

* Use 0.5 ml if using rotor-stator.

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Purifying RNA from Animal Tissues, continued

Summary of Lysis Methods

The following table provides a summary of lysis methods based on sample type and size. **Note:** When lysing ≥10 mg of frozen or fresh fibrous tissue with a mortar and pestle, use liquid nitrogen.

Tissue type	Sample size	Available lysis methods
Frozen or Fresh fibrous	≤10 mg	• Pestle/microcentrifuge tube • Rotor-stator
	10–200 mg	• Pestle/mortar with liquid nitrogen • Rotor-stator
Fresh soft	≤100 mg	• Pestle/microcentrifuge tube • Rotor-stator
	100–200 mg	• Rotor-stator

Lysis and Homogenization: ≤10 mg Frozen or Fresh Fibrous Tissue

Use one of the following protocols below to prepare ≤10 mg of frozen tissue or fresh fibrous tissue.

Pestle:

1. Transfer tissue to a 1.5-ml tube cooled on ice. Immediately add 0.3-ml of RNA Lysis Solution prepared with 2-mercaptoethanol (see page 12).
2. Mince tissue using an RNase-free pestle in up/down and twisting movements in the tube until tissue is thoroughly disrupted and lysed.
3. Proceed with **one** of the following homogenization options at room temperature:
   - Transfer lysate to the Homogenizer inserted in an RNase-free tube and centrifuge at 12,000 × *g* for 2 minutes. Remove the Homogenizer from the tube.
   - Pass the lysate 5–10 times through an 18–21-gauge needle attached to an RNase-free syringe, and then centrifuge at 12,000 × *g* for 2 minutes. Transfer the supernatant to a new RNase-free tube.

Proceed to **Binding, Washing, and Elution**, page 16.

Rotor-stator:

1. Transfer tissue to a 4-ml round-bottom tube on ice. Immediately add 0.5 ml of RNA Lysis Solution prepared with 2-mercaptoethanol (see page 12).
2. Homogenize sample for 30–40 seconds using a rotor-stator.
3. Centrifuge at ~2,600 × *g* for 5 minutes at room temperature.
4. Carefully transfer the supernatant to a clean RNase-free tube.

Proceed to **Binding, Washing, and Elution**, page 16.

Continued on next page

Lysis and Homogenization: 10–100 mg Frozen or Fresh Fibrous Tissue

Use one of the following protocols to prepare 10–100 mg of frozen tissue or fresh fibrous tissue.

Pestle:

1. Add liquid nitrogen to an RNase-free mortar and grind tissue thoroughly using an RNase-free pestle.
2. Transfer tissue powder to an RNase-free 2-ml round-bottom microcentrifuge tube that has been cooled on liquid nitrogen. Allow liquid nitrogen to evaporate.
3. Immediately add 0.6 ml (for 10–60 mg tissue) or 1.2 ml (for 60–100 mg tissue) of RNA Lysis Solution prepared with 2-mercaptoethanol (see page 12).
4. Proceed with **one** of the following homogenization options at room temperature:
   - Transfer up to 0.6 ml of lysate to the Homogenizer inserted in an RNase-free tube and centrifuge at 12,000 × *g* for 2 minutes. Remove the Homogenizer from the tube.
   - Pass the lysate 5–10 times through an 18–21-gauge needle attached to an RNase-free syringe, and then centrifuge at 12,000 × *g* for 2 minutes. Transfer the supernatant to a new RNase-free tube.

Proceed to **Binding, Washing, and Elution**, page 16.

Rotor-stator:

1. Transfer tissue to a 4-ml round-bottomed tube on ice. Immediately add 0.6 ml (for 10–60 mg tissue) or 1.2 ml (for 60–100 mg tissue) of RNA Lysis Solution prepared with 2-mercaptoethanol (see page 12).
2. Homogenize sample for 30–40 seconds using a rotor-stator.
3. Centrifuge at ~2,600 × *g* for 5 minutes at room temperature.
4. Carefully transfer the supernatant to a clean RNase-free tube.

Proceed to **Binding, Washing, and Elution**, page 16.

Lysis and Homogenization: 100–200 mg Frozen or Fresh Fibrous Tissue

Use the following protocol to prepare 100–200 mg of frozen tissue or fresh fibrous tissue.

1. Transfer tissue to a 15-ml round-bottomed tube on ice. Immediately add 2.4 ml of RNA Lysis Solution prepared with 2-mercaptoethanol (see page 12).
2. Homogenize sample for at least 45 seconds using a rotor-stator at maximum speed.
3. Centrifuge at ~2,600 × *g* for 5 minutes at room temperature.
4. Carefully transfer the supernatant to a clean RNase-free tube.

Proceed to **Binding, Washing, and Elution**, page 16.

Continued on next page

Lysis and Homogenization: ≤100 mg Fresh Soft Tissue

Use one of the following protocols to prepare up to 100 mg of fresh soft tissue.

Pestle:

1. Transfer tissue to a 1.5-ml or 2.0-ml round-bottomed tube. Immediately add the appropriate volume of RNA Lysis Solution prepared with 2-mercaptoethanol (see page 12):

Amount of tissue	Volume of RNA Lysis Solution
≤10 mg	0.3 ml
10–60 mg	0.6 ml
60–100 mg	1.2 ml

2. Mince tissue using an RNase-free pestle that fits to the shape of the tube bottom in up/down and twisting movements until the tissue is thoroughly disrupted and lysed.
3. Centrifuge at 12,000 × *g* for 2 minutes at room temperature. Transfer supernatant to a clean RNase-free tube.
4. Proceed with **one** of the following homogenization options at room temperature:
   - Transfer lysate to the Homogenizer inserted in an RNase-free tube and centrifuge at 12,000 × *g* for 2 minutes. Remove the Homogenizer from the tube.
   - Pass the lysate 5–10 times through an 18–21-gauge needle attached to an RNase-free syringe, and then centrifuge at 12,000 × *g* for 2 minutes. Transfer the supernatant to a new RNase-free tube.

Proceed to **Binding, Washing, and Elution**, page 16.

Rotor-stator:

1. Transfer tissue to a 4-ml round-bottomed tube. Immediately add 0.6 ml (for ≤60 mg tissue) or 1.2 ml (for 60–100 mg tissue) of RNA Lysis Solution prepared with 2-mercaptoethanol (see page 12).
2. Quickly homogenize the sample using a rotor-stator at maximum speed for at least 45 seconds.
3. Centrifuge at ~2,600 × *g* for 5 minutes at room temperature.
4. Transfer the supernatant to a clean RNase-free tube.

Proceed to **Binding, Washing, and Elution**, page 16.

Lysis and Homogenization: 100–200 mg Fresh Soft Tissue

Use the following protocol to prepare 100–200 mg of soft fresh tissue.

1. Transfer tissue to a 15-ml round-bottomed tube. Immediately add 2.4 ml of RNA Lysis Solution prepared with 2-mercaptoethanol (see page 12).
2. Quickly homogenize the sample using a rotor-stator at maximum speed for at least 45 seconds.
3. Centrifuge at ~2,600 × *g* for 5 minutes at room temperature.
4. Transfer the supernatant to a clean RNase-free tube.

Proceed to **Binding, Washing, and Elution**, page 16.

Continued on next page

Binding, Washing, and Elution

Follow the steps below to bind, wash, and elute your tissue sample, prepared as described on the previous pages:

1. Add one volume of 70% ethanol to each volume of tissue homogenate.
2. Mix thoroughly by shaking or vortexing. Disperse any visible precipitate that may form after adding ethanol.
3. Transfer up to 700 µl of the sample (including any remaining precipitate) to the RNA Spin Cartridge pre-inserted in a collection tube.
4. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.
5. Repeat Steps 3–4 until the entire sample has been processed.

   ***Optional*:** If your downstream application requires DNA-free total RNA, you may use the convenient on-column DNase I treatment at this point in the procedure (see page 38).
6. Add 700 µl of Wash Buffer I to the spin cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through and the collection tube.
7. Place the spin cartridge into a clean RNA Wash Tube, provided in the kit.
8. Add 500 µl Wash Buffer II with ethanol (prepared as described on page 7) to the spin cartridge.
9. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.
10. Repeat Steps 8–9 once.
11. Centrifuge the spin cartridge at 12,000 × *g* for 1 minute at room temperature to dry the membrane with attached RNA.
12. Discard the collection tube, and insert the cartridge into an RNA Recovery Tube supplied with the kit.
13. To elute the RNA, add 30–100 µl of RNase-free water to the center of the spin cartridge, and incubate at room temperature for 1 minute. See page 7 for detailed elution parameters.
12. Centrifuge the spin cartridge for 2 minutes at ≥12,000 × *g* at room temperature. If you are performing sequential elutions, collect all elutes into the same tube.

To determine the quantity and quality of the RNA, see page 30. To perform DNase I digestion of the purified RNA, see page 39. If you will use the RNA within a few hours, store on ice. For longer storage, store at –80°C.

Purifying RNA from Plant Tissues

Introduction

This section provides protocols for purifying total RNA from up to 250 mg of fresh or frozen plant tissue.

Note

- Protocols for frozen tissue/fibrous fresh tissue are provided starting on the next page. Protocols for soft fresh tissue are provided starting on page 15.
- For samples that are difficult to lyse, you can use TRIzol® Reagent followed by purification using the PureLink™ Micro-to-Midi Total RNA System. See the protocol on page 36.

Important

- Frozen tissue must remain frozen at –80°C prior to lysis. Cool tubes in dry ice before placing frozen tissue in them. Thawing of frozen tissue prior to lysis may result in RNA degradation and loss of RNA yield.
- For plant tissues rich in polyphenolics or starch (*e.g.*, pine needles, potato tubers), we recommend using the PureLink™ Plant RNA Reagent for isolation of RNA (see page vi for ordering information). For more details, visit our Web site at www.invitrogen.com or contact Technical Service (page 42).
- Fast and complete disruption of tissue during the lysis step is important to prevent RNA degradation.

Additional Materials Needed

You will need the following additional items not provided in the kit:

- 2-mercaptoethanol
- 96–100% ethanol
- RNase-free pipette tips
- Round-bottomed RNase-free tubes (1.5 ml–15 ml, depending on amount of tissue)
- Microcentrifuge capable of centrifuging 12,000 × *g*
- **For fresh fibrous or frozen tissue:** Pestle/mortar and liquid nitrogen
- **For fresh fibrous or fresh soft tissue:** RNase-free scalpels and tweezers, petri dish
- Homogenizer ***or*** rotor-stator homogenizer (see page 6)

Preparing RNA Lysis Solution

Prepare the RNA Lysis Solution **fresh** for each use by adding 1% (v/v) 2-mercaptoethanol (*e.g.*, add 10 µl of 2-mercaptoethanol to every 1 ml of RNA Lysis Solution). Use the table below to determine the amount of RNA Lysis Solution needed per sample.

Amount of tissue	RNA Lysis Solution (ml)
≤100 mg	0.5
100–250 mg	1.0

Continued on next page

Purifying RNA from Plant Tissues, continued

Summary of Lysis Methods

The following table provides a summary of lysis methods based on sample type.

Tissue type	Recommended lysis methods
Frozen or Fresh fibrous	Pestle/mortar with liquid nitrogen
Fresh soft	Rotor-stator

Lysis and Homogenization: Frozen or Fresh Fibrous Tissue

Use the following protocol to prepare frozen tissue or fresh fibrous tissue.

1. **Fresh fibrous tissue only:** On ice, quickly cut tissue into small, ≤0.5-cm^2 pieces using an RNase-free scalpel and tweezers.
2. Add liquid nitrogen to an RNase-free mortar and grind frozen or fresh fibrous tissue thoroughly using an RNase-free pestle.
3. Transfer tissue powder to an RNase-free, appropriately sized round-bottom microcentrifuge tube that has been cooled on liquid nitrogen. Allow liquid nitrogen to evaporate.
4. Immediately add 0.5 ml (for ≤100 mg tissue) or 1.0 ml (for 100–250 mg tissue) of RNA Lysis Solution prepared with 2-mercaptoethanol (see page 17).
5. Proceed with **one** of the following homogenization options at room temperature:
   - Vortex the lysate to disperse, and incubate for 3 minutes at room temperature. Then transfer up to 0.5 ml of lysate to the Homogenizer inserted in an RNase-free tube and centrifuge at 12,000 × *g* for 2 minutes. Use additional Homogenizers for each 0.5 ml of sample. When done, remove the Homogenizer from the tube.
   - Homogenize sample for at least 45 seconds using a rotor-stator at maximum speed. Then centrifuge at ~2,600 × *g* for 5 minutes at room temperature. Carefully transfer the supernatant to a clean RNase-free tube.

Proceed to **Binding, Washing, and Elution**, page 19.

Lysis and Homogenization: Fresh Soft Tissue

Use the following protocol to prepare soft fresh tissue.

1. On ice, quickly cut tissue into small, ≤0.5-cm^2 pieces using an RNase-free scalpel and tweezers. Transfer tissue to an appropriately sized round-bottomed microcentrifuge tube.
2. Immediately add 0.5 ml (for ≤100 mg tissue) or 1.0 ml (for 100–250 mg tissue) of RNA Lysis Solution prepared with 2-mercaptoethanol (see page 17).
3. Quickly homogenize the sample using a rotor-stator at maximum speed for at least 45 seconds.
4. Centrifuge at ~2,600 × *g* for 5 minutes at room temperature.
5. Transfer the supernatant to a clean RNase-free tube.

Proceed to **Binding, Washing, and Elution**, page 19.

Continued on next page

Purifying RNA from Plant Tissues, continued

Binding, Washing, and Elution

Follow the steps below to bind, wash, and elute your plant tissue sample, prepared as described on the previous page:

1. Add 0.5 volume of 96–100% ethanol to each volume of tissue homogenate.
2. Mix thoroughly by shaking or vortexing. Disperse any visible precipitate that may form after adding ethanol.
3. Transfer up to 700 µl of the sample (including any remaining precipitate) to the RNA Spin Cartridge pre-inserted in a collection tube.
4. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.
5. Repeat Steps 3–4 until the entire sample has been processed.

   ***Optional*:** If your downstream application requires DNA-free total RNA, you may use the convenient on-column DNase I treatment at this point in the procedure (see page 38).
6. Add 700 µl of Wash Buffer I to the spin cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through and the collection tube.
7. Place the spin cartridge into a clean RNA Wash Tube, provided in the kit.
8. Add 500 µl of Wash Buffer II with ethanol (prepared as described on page 7) to the spin cartridge.
9. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.
10. Repeat Steps 8–9 once.
11. Centrifuge the spin cartridge at 12,000 × *g* for 1 minute at room temperature to dry the membrane with attached RNA.
12. Discard the collection tube, and insert the cartridge into an RNA Recovery Tube supplied with the kit.
13. To elute the RNA, add 30–100 µl of RNase-free water to the center of the spin cartridge, and incubate at room temperature for 1 minute. See page 7 for detailed elution parameters.
14. Centrifuge the spin cartridge for 2 minutes at ≥12,000 × *g* at room temperature. If you are performing sequential elutions, collect all elutes into the same tube.

To determine the quantity and quality of the RNA, see page 30. To perform DNase I digestion of the purified RNA, see page 39. If you will use the RNA within a few hours, store on ice. For longer storage, store at –80°C.

Purifying RNA from Whole Blood

Introduction

This section provides instructions for preparing total RNA from up to 0.2 ml of fresh or frozen whole blood.

Important

We recommend collecting whole blood in the presence of anticoagulants such as EDTA or sodium citrate and store at 4ºC until use. Freshly drawn blood can be used without anticoagulants. You may also process frozen blood, however, yield and quality of RNA might be lower.

Materials Needed

You will need the following items in addition to the components provided in the kit:

- 2-mercaptoethanol
- 100% ethanol
- Microcentrifuge capable of centrifuging 12,000 × *g*
- 1.5-ml RNase-free microcentrifuge tubes
- RNase-free pipette tips

Preparing RNA Lysis Solution

Prepare the RNA Lysis Solution fresh for each use by adding 1% (v/v) 2-mercaptoethanol. To prepare enough solution to purify ≤0.2 ml of whole blood, add 2 µl of 2-mercaptoethanol to 0.2 ml of RNA Lysis Solution.

Isolating RNA from Whole Blood

Use the following protocol to purify total RNA from 0.2 ml of fresh whole blood:

1. In a 1.5-ml RNase-free microcentrifuge tube, add 0.2 ml of RNA Lysis Solution prepared with 2-mercaptoethanol (see above) to ≤0.2 ml of whole blood sample.
2. Vortex thoroughly to disrupt and lyse blood cells, and centrifuge lysate at 12,000 × *g* for 2 minutes at room temperature.
3. Transfer supernatant to clean 1.5-ml microcentrifuge tube.
4. Add 200 µl of 100% ethanol to the tube, and disperse any precipitate by vortexing or pipetting up and down several times.
5. Transfer the sample (including any remaining precipitate) to the RNA Spin Cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through.

   ***Optional*:** If your downstream application requires DNA-free total RNA, you may use the convenient on-column DNase I treatment at this point in the procedure (see page 38).
6. Add 700 µl of Wash Buffer I to the spin cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through and the collection tube.

Procedure continued on the next page

Continued on next page

Purifying RNA from Whole Blood, continued

Isolation of RNA from Whole Blood, continued

Procedure continued from the previous page.

7. Place the spin cartridge into a clean RNA Wash Tube, provided in the kit.
8. Add 500 µl of Wash Buffer II with ethanol (prepared as described on page 7) to the spin cartridge.
9. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.
10. Repeat Steps 8–9 once.
11. Centrifuge the spin cartridge at 12,000 × *g* for 1 minute at room temperature to dry the membrane with attached RNA.
12. Discard the collection tube, and insert the cartridge into an RNA Recovery Tube supplied with the kit.
13. To elute the RNA, add 30–100 µl of RNase-free water to the center of the spin cartridge, and incubate at room temperature for 1 minute. See page 7 for detailed elution parameters.
14. Centrifuge the spin cartridge for 2 minutes at ≥12,000 × *g* at room temperature. If you are performing sequential elutions, collect all elutes into the same tube.

To determine the quantity and quality of the RNA, see page 30. To perform DNase I digestion of the purified RNA, see page 39. If you will use the RNA within a few hours, store on ice. For longer storage, store at –80°C.

Purifying RNA from Yeast Cells

Introduction

This section provides instructions for preparing total RNA from up to 5×10^8 yeast cells. The protocol includes two different options for disrupting yeast cells: enzymatic lysis and mechanical lysis. Enzymatic lysis is more convenient but is not practical for kinetic experiments. Mechanical lysis is suitable for kinetic experiments but is more labor-intensive.

Note

- Grow yeast cells overnight prior to performing the purification procedures on the following pages.
- Fast and complete disruption of the cells is important to prevent degradation of RNA prior to purification for optimal yields of total RNA.

Materials Needed

You will need the following items in addition to the components provided in the kit:

- 2-mercaptoethanol
- Microcentrifuge capable of centrifuging 12,000 × *g*
- 1.5-ml RNase-free microcentrifuge tubes
- RNase-free pipette tips
- Homogenizer (see page 6) ***or*** RNase-free syringe (1 ml) with 18-21 gauge needle ***or*** rotor-stator homogenizer (see page 6)
- 4–15 ml round-bottomed RNase-free tubes (if using rotor-stator)
- **For enzymatic lysis:**
  96–100% ethanol
  Zymolase (Zymolyase™, Lyticase)
- **For mechanical lysis:**
  Mortar (5-cm diameter) and pestle
  Dry ice (10 g/sample)

Preparing RNA Lysis Solution

Prepare the RNA Lysis Solution fresh for each use by adding 1% (v/v) 2-mercaptoethanol (*e.g.,* add 10 µl of 2-mercaptoethanol to every 1 ml of RNA Lysis Solution). Use the table below to determine the amount of RNA Lysis Solution needed for each lysis method.

Lysis method	RNA Lysis Solution (ml)
Mechanical	0.5 ml
Enzymatic	200 µl

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Purifying RNA from Yeast Cells, continued

Lysis and Homogenization: Enzymatic Disruption

Use the following protocol to prepare yeast cells using enzymatic disruption.

Note: Enzymatic disruption is **not recommended** for kinetic experiments.

1. Prepare a digestion buffer with zymolase following the manufacturer's instructions or use the digestion buffer provided by the supplier.

   Note: The amount of digestion buffer and zymolase depends on the yeast cells type and sample size. Follow the manufacturer's instructions.
2. Transfer ≤5 x 10^8 log-phase yeast cells to an appropriately sized microcentrifuge tube and centrifuge at 500 × *g* for 5 minutes at 4ºC. Discard the supernatant.
3. Add up to 100 µl of zymolase digestion buffer to the yeast cell pellet.
4. Incubate the tube for 30–60 minutes at 30ºC. The incubation time may vary depending on the yeast cell type and enzyme used.
5. Add 300 µl of RNA Lysis Solution prepared with 2-mercaptoethanol (see previous page) to the tube.
6. Thoroughly mix by vortexing.
7. Pellet cells at 12,000 × *g* for 2 minutes at room temperature.
8. Transfer supernatant to a clean RNase-free microcentrifuge.

Proceed to **Binding, Washing, and Elution**, page 24.

Lysis and Homogenization: Mechanical Disruption

Use the following protocol to prepare yeast cells using mechanical disruption.

1. Transfer ≤5 x 10^8 log-phase yeast cells to an appropriately sized microcentrifuge tube and centrifuge at 500 × *g* for 5 minutes at 4ºC. Discard the supernatant.
2. Crush approximately 10 grams of dry ice in a mortar to a powder.
3. Add 0.5 ml of RNA Lysis Solution prepared with 2-mercaptoethanol (see previous page) to the yeast cells from Step 1, and thoroughly resuspend.
4. Add the suspension drop-wise onto the crushed dry ice. Grind the mixture with a pestle until the dry ice has evaporated and the paste begins to melt.
5. Homogenize the lysate using **one** of the following options:
   - Transfer the melted paste to a Homogenizer inserted in an RNase-free tube, and centrifuge at 12,000 × *g* for 2 minutes at room temperature. Remove the Homogenizer cartridge from the tube.
   - Transfer the melted paste to a 1.5-ml RNase-free microcentrifuge tube and pass the lysate 5 times through an 18–21-gauge needle attached to an RNase-free syringe. Centrifuge at 12,000 × *g* for 2 minutes at room temperature, and transfer the supernatant to a clean RNase-free microcentrifuge tube.
   - Transfer the melted paste to a 4–15 ml round-bottomed microcentrifuge tube and homogenize using a rotor-stator homogenizer at maximum speed for at least 45 seconds. Centrifuge homogenate at ~2,600 × *g* for 5 minutes at room temperature, and transfer supernatant to clean RNase-free microcentrifuge tube.

Proceed to **Binding, Washing, and Elution**, page 24.

Continued on next page

Binding, Washing, and Elution

Follow the steps below to bind, wash, and elute your yeast cell sample, prepared as described on the previous page. Note the different ethanol requirements depending on whether you performed enzymatic or mechanical disruption of the cells:

1. **Enzymatic Disruption:** Add 220 µl of 96–100% ethanol to each volume of yeast cell homogenate prepared by enzymatic disruption.

   Mechanical Disruption: Add 1 volume of 70% ethanol to each volume of yeast cell homogenate prepared by mechanical disruption.
2. Mix thoroughly by vortexing. Disperse any visible precipitate that may form after adding ethanol.
3. Transfer up to 500 µl of the sample (including any remaining precipitate) to the RNA Spin Cartridge pre-inserted in a collection tube.
4. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.
5. Repeat Steps 3–4 until the entire sample has been processed.

   ***Optional*:** If your downstream application requires DNA-free total RNA, you may use the convenient on-column DNase I treatment at this point in the procedure (see page 38).
6. Add 700 µl of Wash Buffer I to the spin cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through and the collection tube.
7. Place the spin cartridge into a clean RNA Wash Tube, provided in the kit.
8. Add 500 µl of Wash Buffer II with ethanol (prepared as described on page 7) to the spin cartridge.
9. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.
10. Repeat Steps 8–9 once.
11. Centrifuge the spin cartridge at 12,000 × *g* for 1 minute at room temperature to dry the membrane with attached RNA.
12. Discard the collection tube, and insert the cartridge into an RNA Recovery Tube supplied with the kit.
13. To elute the RNA, add 30–100 µl of RNase-free water to the center of the spin cartridge, and incubate at room temperature for 1 minute. See page 7 for detailed elution parameters.
14. Centrifuge the spin cartridge for 2 minutes at ≥12,000 × *g* at room temperature. If you are performing sequential elutions, collect all elutes into the same tube.

To determine the quantity and quality of the RNA, see page 30. To perform DNase I digestion of the purified RNA, see page 39. If you will use the RNA within a few hours, store on ice. For longer storage, store at –80°C.

Purifying RNA from Bacterial Cells

Introduction

This section provides instructions for preparing total RNA from up to 1×10^9 bacterial cells.

Materials Needed

You will need the following items in addition to the components provided in the kit:

- 2-mercaptoethanol
- 100% ethanol
- 10% SDS (in RNase-free water), 0.5 µl/sample
- Lysozyme buffer, 100 µl/sample: 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 10 mg/ml lysozyme (in RNase-free water)
- Microcentrifuge capable of centrifuging 12,000 × *g*
- 1.5-ml RNase-free microcentrifuge tubes
- RNase-free pipette tips
- Homogenizer (see page 6) ***or*** RNase-free syringe (1 ml) with 18-21 gauge needle ***or*** rotor-stator homogenizer (see page 6)

Note

- For best results, use cells in log-phase growth for isolation of RNA.
- Fast and complete disruption of the cells is important to prevent degradation of RNA prior to purification for optimal yields of total RNA.

Preparing Lysozyme and RNA Lysis Solutions

Lysozyme Solution:

For each sample of up to 1×10^9 bacterial cells, prepare 100 µl of lysozyme solution containing:

- 10 mM Tris-HCl (pH 8)
- 0.1 mM EDTA
- 1 mg lysozyme

RNA Lysis Solution:

Prepare the RNA Lysis Solution fresh for each use by adding 1% (v/v) 2-mercaptoethanol (*e.g.*, add 10 µl of 2-mercaptoethanol to every 1 ml of RNA Lysis Solution). Use 350 µl of freshly prepared RNA Lysis Solution for ≤1×10^9 bacterial cells.

Important

Make sure that the pH of the lysozyme buffer is correct. Lower pH may result in reduced RNA yield.

Continued on next page

Purifying RNA from Bacterial Cells, continued

Lysis and Homogenization

Use the following protocol to prepare bacterial cells.

1. Harvest ≤1 × 10^9 log-phase bacterial cells, transfer them to an appropriately sized microcentrifuge tube, and centrifuge at 500 × *g* for 5 minutes at 4ºC to pellet. Discard the supernatant.
2. Add 100 µl of prepared lysozyme solution (see previous page) to the cell pellet, and resuspend by vortexing.
3. Add 0.5 µl of 10% SDS solution, and mix well by vortexing.
4. Incubate the tube for 5 minutes at room temperature.
5. Add 350 µl of RNA Lysis Solution prepared with 2-mercaptoethanol (see previous page), and mix well by vortexing.
6. Homogenize the cell lysate using **one** of the following options:
   - Transfer the lysate to a Homogenizer inserted in an RNase-free tube, and centrifuge at 12,000 × *g* for 2 minutes at room temperature. Remove the Homogenizer cartridge from the tube.
   - Transfer the lysate to a 1.5-ml RNase-free microcentrifuge tube and pass 5 times through an 18–21-gauge needle attached to an RNase-free syringe. Centrifuge at 12,000 × *g* for 2 minutes at room temperature, and transfer the supernatant to a clean RNase-free microcentrifuge tube.
   - Transfer the lysate to a 4–15 ml round-bottomed microcentrifuge tube and homogenize using a rotor-stator homogenizer at maximum speed for at least 45 seconds. Centrifuge homogenate at ~2,600 × *g* for 5 minutes at room temperature, and transfer supernatant to clean RNase-free microcentrifuge tube.

Proceed to **Binding, Washing, and Elution**, below.

Binding, Washing, and Elution

Follow the steps below to bind, wash, and elute your bacterial cell sample, prepared as described above:

1. Add 250 µl of 100% ethanol to each volume of bacterial cell homogenate.
2. Mix thoroughly by vortexing. Disperse any visible precipitate that may form after adding ethanol.
3. Transfer the sample (including any remaining precipitate) to the RNA Spin Cartridge pre-inserted in a collection tube.
4. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.

   ***Optional*:** If your downstream application requires DNA-free total RNA, you may use the convenient on-column DNase I treatment at this point in the procedure (see page 38).
5. Add 700 µl of Wash Buffer I to the spin cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through and the collection tube.

Procedure continued on the next page.

Continued on next page

Purifying RNA from Bacterial Cells, continued

Binding, Washing, and Elution, continued

Procedure continued from the previous page.

6. Place the spin cartridge into a clean RNA Wash Tube, provided in the kit.
7. Add 500 µl of Wash Buffer II with ethanol (prepared as described on page 7) to the spin cartridge.
8. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.
9. Repeat Steps 7–8 once.
10. Centrifuge the spin cartridge at 12,000 × *g* for 1 minute at room temperature to dry the membrane with attached RNA.
11. Discard the collection tube, and insert the cartridge into an RNA Recovery Tube supplied with the kit.
12. To elute the RNA, add 30–100 µl of RNase-free water to the center of the spin cartridge, and incubate at room temperature for 1 minute. See page 7 for detailed elution parameters.
13. Centrifuge the spin cartridge for 2 minutes at ≥12,000 × *g* at room temperature. If you are performing sequential elutions, collect all elutes into the same tube.

To determine the quantity and quality of the RNA, see page 30. To perform DNase I digestion of the purified RNA, see page 39. If you will use the RNA within a few hours, store on ice. For longer storage, store at –80°C.

Purifying RNA from Liquid Samples

Introduction

This section provides instructions for preparing total RNA from up to 1.2 ml of liquid sample.

Liquid Sample Types

The types of liquid sample supported by this kit include enzymatic reactions (DNase digestion, RNA labeling), cytoplasmic RNA extracts from mammalian cells, and *in vitro* transcription reactions (Sambrook *et al.*, 1989). This kit can also be used to clean up liquid RNA samples (*e.g.*, desalting).

Materials Needed

You will need the following items in addition to the components provided in the kit:

- 2-mercaptoethanol
- 96–100% ethanol
- Microcentrifuge capable of centrifuging 12,000 × *g*
- 1.5-ml RNase-free microcentrifuge tubes
- RNase-free pipette tips

Preparing RNA Lysis Solution

Prepare the RNA Lysis Solution fresh for each use by adding 1% (v/v) 2-mercaptoethanol (*e.g.*, add 10 µl of 2-mercaptoethanol to every 1 ml of RNA Lysis Solution). Use 1 volume of freshly prepared RNA Lysis Solution for each volume of liquid sample.

Isolation of RNA from Liquid Samples

Use the following protocol to purify total RNA from liquid samples:

1. To one volume of liquid sample (≤1.2 ml), add one volume of RNA Lysis Solution prepared with 2-mercaptoethanol (see above) followed by the same volume of 96–100% ethanol (*e.g.*, to 1 ml of sample, add 1 ml of RNA Lysis Solution followed by 1 ml of ethanol).
2. Mix by vortexing or pipetting up and down 5 times.
3. Transfer up to 700 µl of the sample to the RNA Spin Cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through and re-insert the cartridge in the tube.
4. Repeat Step 3 until the entire sample is processed.

   ***Optional*:** If your downstream application requires DNA-free total RNA, you may use the convenient on-column DNase I treatment at this point in the procedure (see page 38).
5. **For cytoplasmic RNA extracts:** Add 700 µl of Wash Buffer I to the spin cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through and the collection tube, and place the spin cartridge into a clean RNA Wash Tube provided in the kit.

   For other samples: Proceed directly to Step 6.

Protocol continued on next page

Continued on next page

Purifying RNA from Liquid Samples, continued

Isolation of RNA from Liquid Samples, continued

Protocol continued from previous page

6. Add 500 µl of Wash Buffer II with ethanol (prepared as described on page 7) to the spin cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.
7. Repeat Step 6 once.
8. Centrifuge the spin cartridge at 12,000 × *g* for 1 minute at room temperature to dry the membrane with attached RNA.
9. Discard the collection tube, and insert the cartridge into an RNA Recovery Tube supplied with the kit.
10. To elute the RNA, add 30–100 µl of RNase-free water to the center of the spin cartridge, and incubate at room temperature for 1 minute. See page 7 for detailed elution parameters.
11. Centrifuge the spin cartridge for 2 minutes at ≥12,000 × *g* at room temperature. If you are performing sequential elutions, collect all elutes into the same tube.

To determine the quantity and quality of the RNA, see page 30. To perform DNase I digestion of the purified RNA, see page 39. If you will use the RNA within a few hours, store on ice. For longer storage, store at –80°C.

Analyzing RNA Yield and Quality

Introduction

After you have purified the total RNA, determine the quantity and quality as described in this section.

RNA Yield

Total RNA is easily quantitated using the Quant-iT™ RiboGreen® RNA Assay Kit or UV absorbance at 260 nm.

Quant-iT™ RiboGreen® RNA Assay Kit

The Quant-iT™ RNA Assay Kit (catalog no. Q-33140; see page vi for ordering information) provides a rapid, sensitive, and specific method for RNA quantitation with minimal interference from DNA, protein, or other common contaminants that affect UV absorbance readings.

The kit contains a state-of-the-art quantitation reagent and pre-diluted standards for standard curve. The assay is performed in a microtiter plate format and is designed for reading in standard fluorescent microplate readers.

UV Absorbance

To determine the quantity by UV absorbance:

1. Dilute an aliquot of the total RNA sample in 10 mM Tris-HCl, pH 7.5. Mix well. Transfer to a cuvette (1-cm path length).

   Note: The RNA must be in a neutral pH buffer to accurately measure the UV absorbance.

2. Determine the OD_{260} of the solution using a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.5.

Calculate the amount of total RNA using the following formula:

Total RNA (µg) = OD_{260} × [40 µg/(1 OD_{260} × 1 ml)] × dilution factor × total sample volume (ml)

Example:

Total RNA was eluted in water in a total volume of 150 µl. A 40-µl aliquot of the eluate was diluted to 500 µl in 10 mM Tris-HCl, pH 7.5. An OD_{260} of 0.188 was obtained. The amount of RNA in the sample is determined as shown below:

Total RNA (µg) = 0.188 × [40 µg/(1 OD_{260} × 1 ml)] × 12.5 × 0.15 = 14.1 µg

RNA Quality

Typically, total RNA isolated using the PureLink™ Micro-to-Midi Total RNA Purification Kit has an $OD_{260/280}$ of >1.8 when samples are diluted in Tris-HCl (pH 7.5). An $OD_{260/280}$ of >1.8 indicates that RNA is reasonably clean of proteins and other UV chromophores that could either interfere with downstream applications or negatively affect the stability of the stored RNA.

RNA quality may also be assessed using a bioanalyzer (see the next page).

Agarose gel electrophoresis of RNA isolated using the PureLink™ kit shows the 28S to 18S band ratio to be >1.5. RNA is judged to be intact if discreet 28S and 18S ribosomal RNA bands are observed.

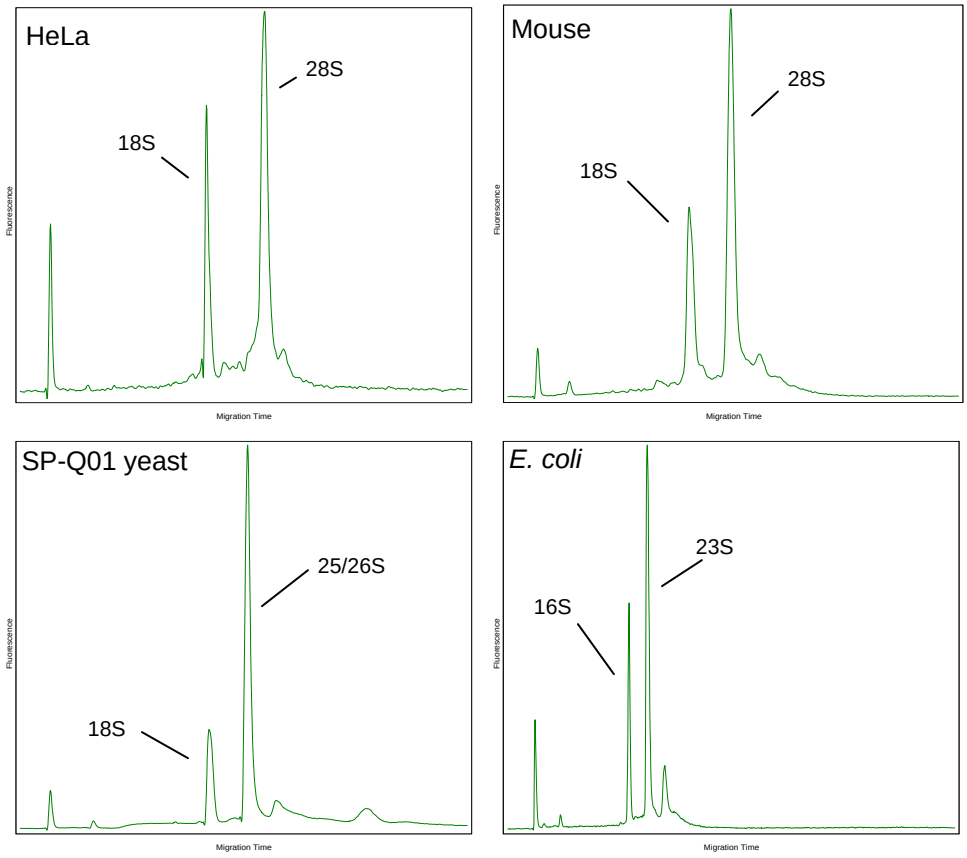
Continued on next page

Analyzing RNA Yield and Quality, continued

Bioanalyzer Analysis of RNA Quality

The quality of purified total RNA can be analyzed using a bioanalyzer such as the Agilent 2100 bioanalyzer with an RNA LabChip®. In the example below, the bioanalyzer was used to show the presence of 18 and 28 S rRNA (mammalian), 18S and 25/26S rRNA (yeast), and 16S and 23S rRNA (bacteria), as well as small RNA species in total RNA purified using the PureLink™ Micro-to-Midi Total RNA Purification System.

Total RNA was purified from HeLa cells, mouse liver, SP-Q01 yeast cells, and *E. coli* bacterial cells using the protocols described in this manual. Aliquots of 2% of the final elution volumes were subjected to bioanalysis using the Agilent 2100 bioanalyzer.



Expected Results

Expected Yields

The following table lists the average yields of total RNA obtained from various samples using the PureLink™ Micro-to-Midi Total RNA Purification System. RNA quantitation was performed using UV absorbance at 260 nm.

Sample type	Sample	Amount	Average Yield (µg)
Animal Cells	HeLa cells	1×10^6	20
		1×10^7	160
		1×10^8	1,000
Animal Tissue	Rat liver	1 mg	5
		10 mg	60
		100 mg	300
		200 mg	650
	Rat brain	1 mg	0.6
		10 mg	6
		100 mg	90
		200 mg	230
	Rat spleen	1 mg	6
		10 mg	58
		100 mg	320
		200 mg	450
	Calf thymus	1 mg	3.2
		10 mg	48
		100 mg	350
		200 mg	ND
Plants leaf	Arabidopsis	100 mg	26
	Wheat	100 mg	31
	Corn	100 mg	36
	Rice	100 mg	37
	Alfalfa	100 mg	32
	Soybean	100 mg	38
	Sugar beet	100 mg	31
		200 mg	65
Yeast cells	*S. cerevisiae*	1×10^7	1.8
		5×10^8	125
ND = not determined			

Troubleshooting

Introduction

Refer to the table below to troubleshoot any problems you may encounter with the PureLink™ Micro-to-Midi Total RNA Purification System.

Problem	Cause	Solution
Clogged Homogenizer	Highly viscous lysate (*e.g.,* calf thymus)	Homogenize sample with rotor-stator homogenizer
Clogged RNA Spin Cartridge	Incomplete homogenization or dispersal of precipitate after ethanol addition	• Follow protocol guidelines for each sample type and amount. • Clear homogenate and remove any particulate or viscous material by centrifugation and use only the supernatant for subsequent loading on to the RNA Spin Cartridge. • Completely disperse any precipitate that forms after adding ethanol to the homogenate.
Low DNA yield	Incomplete lysis and homogenization	• Ensure that 10 µl of 2-mercaptoethanol was added per 1 ml of RNA Lysis Solution. • Perform all steps at room temperature unless directed otherwise. • Decrease the amount of starting material used. • Use the proper homogenization methods according to recommendations in the sample-specific protocols. • Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in the RNA Lysis Solution to achieve optimal lysis.
	Poor quality of starting material	• The yield and quality of RNA isolated depends on the type and age of the starting material. • Be sure to use fresh sample and process immediately after collection or freeze the sample at -80ºC or in liquid nitrogen immediately after harvesting.
	Ethanol not added to Wash Buffer II	Be sure that ethanol was added to Wash Buffer II as directed on page 7.
	Incorrect elution conditions	• Add RNase-free water and perform incubation for 1 minute before centrifugation. • Follow the recommendations on page 7 on elution conditions. • To recover more RNA, perform a second elution step.

Continued on next page

Troubleshooting, continued

Problem	Cause	Solution
RNA degraded	RNA contaminated with RNase	• Use RNase-free pipet tips with aerosol barriers. • Change gloves frequently. • Swipe automatic pipettes with RNase AWAY™ solution after washing RNA Spin Cartridge with Wash Buffer I.
	Improper handling of sample from harvest until lysis	• If not processed immediately, quick-freeze tissue immediately after harvesting and store at –80ºC or in liquid nitrogen. • Frozen samples must remain frozen until RNA Lysis Solution was added. • Perform the lysis quickly after adding RNA Lysis Solution
	Tissue very rich in RNases (*e.g.*, rat pancreas)	• RNA isolated from tissue rich in RNases may require the addition of RNAse inhibitors/inactivators (see page vi) to protect the RNA from degradation. • Elute samples in 100% formamide. If the RNA is used for mRNA isolation of Northern blots, elute in 0.1% SDS.
DNA contamination	Incomplete homogenization or incomplete dispersal of precipitate after ethanol addition	• Follow protocol guidelines for each sample type and amount. • Perform optional DNase digestion step during the sample preparation (see protocol page 38) or after purification (see protocol page 39).
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified RNA	Traces of ethanol from the Wash Buffer II can inhibit downstream enzymatic reactions. Discard Wash Buffer II flow through. Place the RNA Spin Cartridge into the Wash Tube and centrifuge the spin cartridge at maximum speed for 2–3 minutes to completely dry the cartridge.
	Presence of salt in purified RNA	Use the correct order of Wash Buffers for washing. Always wash the cartridge with Wash Buffer I followed by washing with Wash Buffer II.
Low A260/280 ratio	Sample was diluted in water; non-buffered water has variable pH (Wilfinger *et al.*, 1997)	Use 10 mM Tris-HCl (pH 7.5) to dilute sample for OD measurements.

Appendix

Using TRIzol® Reagent with the PureLink™ Micro-to-Midi System

Introduction

This section provides instructions for using TRIzol® Reagent in conjunction with the PureLink™ Micro-to Midi RNA Total RNA Purification System to isolate total RNA from samples that are difficult to lyse (*e.g.,* fibrous animal tissues or plant tissue). This combined protocol allows you to purify ultrapure total RNA for sensitive downstream applications such as qPCR or microarray analysis.

To obtain high-quality total RNA, be careful to follow the guidelines on page 4 for handling RNA.

Important

The maximum binding capacity of the PureLink™ Micro-to Midi cartridges is ~1 mg of RNA. If you are processing samples that contain more than 1 mg of total RNA, divide the sample into aliquots that each contains less than 1 mg of total RNA for each cartridge used.

Materials Needed

You will need the following additional items, ***not*** provided in the kit:

- TRIzol® Reagent (see page vi)
- Chloroform
- 2-mercaptoethanol
- 96–100% ethanol or 70% ethanol (in RNase-free water), depending on protocol option used (see **Step 6**, next page)
- Microcentrifuge capable of centrifuging 12,000 × *g*
- 1.5-ml RNase-free microcentrifuge tubes
- RNase-free pipette tips

CAUTION

TRIzol® Reagent is toxic in contact with skin and if swallowed. Causes burns. Please consult the manual of the **TRIzol® Reagent** for further safety instructions.

Continued on next page

Using TRIzol® Reagent with the PureLink™ Micro-to-Midi System, continued

Lysate Preparation with TRIzol® Reagent

Use TRIzol® Reagent to prepare lysates from various sample types as described below. See the manual provided with TRIzol® Reagent for more information.

Tissues

Homogenize tissue samples in 1 ml TRIzol® Reagent per 50–100 mg tissue using a tissue homogenizer or rotor-stator according to the TRIzol® Reagent protocol.

Adherent Cells

Lyse cells directly in a culture dish by adding 1 ml of TRIzol® Reagent to the dish and passing the cell lysate several times through a pipette tip. The amount of TRIzol® Reagent required is based on the culture dish area (1 ml per 10 cm^2) and not on the number of cells present.

Suspension Cells

Harvest cells and pellet by centrifugation. Use 1 ml of TRIzol® Reagent per 5–10 × 10^6 animal, plant, or yeast cells, or 1 × 10^7 bacterial cells. Lyse cells by repetitive pipetting up and down.

Phase Separation with TRIzol® Reagent

Following cell or tissue lysis as described above, perform the following steps to isolate the sample.

1. Incubate the lysate prepared as above at room temperature for 5 minutes to allow complete dissociation of nucleoprotein complexes.
2. Add 0.2 ml chloroform per 1 ml of TRIzol® Reagent used and shake the tube vigorously by hand for 15 seconds.

   Note: Vortexing the sample may increase DNA contamination of your RNA sample. Avoid vortexing if your downstream application is sensitive to presence of DNA or perform a DNase-digestion step on-column (page 38) or after purification (page 39).
3. Incubate at room temperature for 2–3 minutes.
4. Centrifuge the sample at 12,000 × *g* for 15 minutes at 4ºC.

   After centrifugation, the mixture separates into a lower, red phenol-chloroform phase, an interphase, and a colorless upper aqueous phase containing the RNA. The volume of the aqueous upper phase is ~600 µl.
5. Transfer ~400 µl of the colorless, upper phase containing RNA to a fresh tube.
6. Add an equal volume of 70% ethanol to obtain a final ethanol concentration of 35% and mix well by vortexing.
7. Mix thoroughly by inverting the tube. Disperse any visible precipitate that may form after adding ethanol.

Proceed to **Binding, Washing, and Elution** on the next page.

Continued on next page

Using TRIzol® Reagent with the PureLink™ Micro-to-Midi System, continued

Binding, Washing, and Elution

Follow the steps below to bind, wash, and elute your yeast cell sample, prepared as described on the previous page. Note the different ethanol requirements depending on whether you performed enzymatic or mechanical disruption of the cells:

1. Transfer up to 700 µl of the sample (prepared as described on the previous page) to the RNA Spin Cartridge pre-inserted in a collection tube.
2. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.
3. Repeat Steps 1–2 until the entire sample has been processed.

   ***Optional*:** If your downstream application requires DNA-free total RNA, you may use the convenient on-column DNase I treatment at this point in the procedure (see page 38).
4. Add 700 µl of Wash Buffer I to the spin cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through and the collection tube.
5. Place the spin cartridge into a clean RNA Wash Tube, provided in the kit.
6. Add 500 µl of Wash Buffer II with ethanol (prepared as described on page 7) to the spin cartridge.
7. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and re-insert the cartridge in the tube.
8. Repeat Steps 6–7 once.
9. Centrifuge the spin cartridge at 12,000 × *g* for 1 minute at room temperature to dry the membrane with attached RNA.
10. Discard the collection tube, and insert the cartridge into an RNA Recovery Tube supplied with the kit.
11. To elute the RNA, add 30–100 µl of RNase-free water to the center of the spin cartridge, and incubate at room temperature for 1 minute. See page 7 for detailed elution parameters.
12. Centrifuge the spin cartridge for 2 minutes at ≥12,000 × *g* at room temperature. If you are performing sequential elutions, collect all elutes into the same tube.

To determine the quantity and quality of the RNA, see page 30. To perform DNase I digestion of the purified RNA, see page 39. If you will use the RNA within a few hours, store on ice. For longer storage, store at –80°C.

DNase I Treatment during RNA Purification

Introduction

This section provides a protocol for removing DNA from the sample while it is loaded on the PureLink™ Micro-to-Midi spin cartridge. See each sample-specific protocol in this manual for the step at which to perform the following on-column DNase I digestion.

Alternatively, you may perform a DNase I digestion of the RNA sample after purification, as described on the following page. However, this may result in reduced RNA yield.

DNase I, Amplification Grade

The following protocol uses DNase I, Amplification Grade. See page vi for ordering information.

On-Column DNase-treatment

After loading the sample onto the spin cartridge, perform the following DNase I-digestion procedure before proceeding to the wash step in the sample-specific purification protocol (see the sample-specific protocol for more information).

1. Add 350 µl of Wash Buffer I to the spin cartridge.
2. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through and the collection tube.
3. Add the following items supplied with DNase I, Amplification Grade, to a clean, RNase-free microcentrifuge tube:

Component	Volume
DNase Buffer	70 µl
DNase I (1 unit/µl)	10 µl
Final Volume	80 µl

4. Mix gently by inverting the tube. Centrifuge briefly to collect the contents of the tube.
5. Transfer the solution to the center of the spin cartridge, and incubate for 15 minutes at room temperature.
6. Add 350 µl of Wash Buffer I to the spin cartridge. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through.

Proceed with the wash step in the appropriate sample-specific protocol.

DNase I Treatment after RNA Purification

Introduction

This section provides a protocol for removing DNA from the sample following RNA purification. You may also perform an on-column DNase-I digestion during the purification procedure (see previous page).

DNase I, Amplification Grade

The following protocol uses DNase I, Amplification Grade. See page vi for ordering information.

DNase-treatment of purified RNA

Following RNA purification using the PureLink™ Micro-to-Midi Total RNA Purification System, perform the following DNase I digestion procedure:

1. Add the following items to the tube with purified RNA:

Component	Volume
RNA (up to 10 µg/µl)	up to 8 µl
10 X DNase I Buffer	1 µl
RNase-free water	add to 9 µl
DNase I, Amplification Grade (1 unit/µl)	1 µl
Final Volume	10 µl

2. Incubate for 15 minutes at room temperature.
3. Heat-inactivate the DNase I **or** remove the DNase I by purification as follows:

   Heat inactivation:

   Add 1 µl of 25 mM EDTA solution to the reaction and heat at 65ºC for 10 minutes.

   Note: When using DNase I-digested RNA directly in RT-PCR, add the oligo(dT) primer (or gene-specific primers) and the nucleotide mix to the sample ***before*** heat inactivation for more consistent and better RT-PCR products.

   Removal DNase I by Purification:

   Proceed to **Purifying RNA from Liquid Samples**, page 28.

Purifying mRNA from *in vitro* Transcription Reactions

Introduction

This section provides a protocol for purifying mRNA from *in vitro* transcription reactions by digesting the DNA in the reaction.

DNase I, Amplification Grade

The following protocol uses DNase I, Amplification Grade. See page vi for ordering information.

Isolating mRNA from *in vitro* Transcription Reactions

To isolate mRNA from *in vitro* transcription reactions:

1. Add the following to each 8-µl volume of *in vitro* transcription reaction:

10 X DNase I Buffer	1 µl
DNase I, Amplification Grade (1 unit/µl)	1 µl

2. Incubate for 15 minutes at room temperature.

Proceed to **Purifying RNA from Liquid Samples**, page 28.

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