

USER GUIDE

ambion[®]
by *life* technologies™

PureLink[®] RNA Mini Kit

For purification of total RNA from a large
variety of samples

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therapeutic or diagnostic use.**

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Kit Contents and Storage

Types of Kits

The PureLink® RNA Mini Kit is available in two sizes:

Product	Cat. no.	Quantity
PureLink® RNA Mini Kit	12183018A	50 preps
	12183025	250 preps

Shipping and Storage

All contents of the PureLink® RNA Mini Kit are shipped at room temperature.

Upon receipt, store all contents at room temperature. Kit contents are stable for up to six months, when properly stored.

Kit Contents

The components included in the PureLink® RNA Mini Kit are listed below. Sufficient reagents are included in the kit to perform 50 preparations (Cat. no. 12183018A) or 250 preparations (Cat. no. 12183025).*

*If your sample contains more than an average amount of RNA, or if you are using a rotor-stator homogenizer, you may need greater volumes of Lysis Buffer than is provided in the PureLink® RNA Mini kit. If extra buffer is required for your sample, you can purchase our bulk PureLink® 96 RNA Lysis Buffer (page 67). Refer to your sample-specific protocol to determine the amount of Lysis Buffer needed for each sample type and amount.

PureLink® RNA Mini Kit Contents	Quantity	
	12183018A	12183025
Lysis Buffer	125 mL	500 mL
Wash Buffer I	50 mL	250 mL
Wash Buffer II	15 mL	75 mL
RNase-Free Water	15.5 mL	75 mL
Spin Cartridges (with collection tubes)	50 each	5 × 50 each
Collection Tubes	50 each	5 × 50 each
Recovery Tubes	50 each	5 × 50 each

Product Use

For research use only. Not for any human or animal therapeutic or diagnostic use.

Introduction

System Description

Kit Usage

The PureLink® RNA Mini Kit provides a simple, reliable, and rapid method for isolating high-quality total RNA from a wide variety of sources, including cells and tissue from animal and plant samples, blood, bacteria, yeast, and liquid samples. The purified total RNA is suitable for use in a variety of downstream applications (see below).

System Overview

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. The sample is then processed through a Spin Cartridge containing a clear silica-based membrane to which the RNA binds. Any impurities are effectively removed by subsequent washing (Vogelstein & Gillespie, 1979). The purified total RNA is then eluted in RNase-Free Water (or Tris Buffer, pH 7.5) and may be used for use in a variety of downstream applications (see below).

Downstream Applications

The purified total RNA eluted using the PureLink® RNA Mini Kit is suitable for use in a variety of applications, including:

- Real-time-PCR (RT-PCR)
 - Real-time quantitative-PCR (qRT-PCR)
 - Northern blotting
 - Nuclease protection assays
 - RNA amplification for microarray analysis
 - cDNA library preparation after poly(A)⁺ selection
-

Continued on next page

System Description, Continued

Advantages of the Kit

The PureLink® RNA Mini Kit offers the following advantages:

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

Starting Material

The various sample types and amounts that can be processed using the PureLink® RNA Mini Kit are listed in the table below:

Sample type	Sample Amount	Page
Animal and plant cells	$\leq 5 \times 10^7$ cells	13
Animal tissue	≤ 200 mg	21
Plant tissue	≤ 250 mg	28
Whole blood	≤ 0.2 mL	34
Yeast cells	$\leq 5 \times 10^8$ cells	37
Bacterial cells	$\leq 1 \times 10^9$ cells	42
Liquid samples*	≤ 1.2 mL	46

*Liquid samples include cytoplasmic RNA extracts from mammalian cells, *in vitro* transcription reactions, PureLink® DNase or DNase I digestions, RNA labeling reactions, and RNA clean-up preps.

Kit Specifications

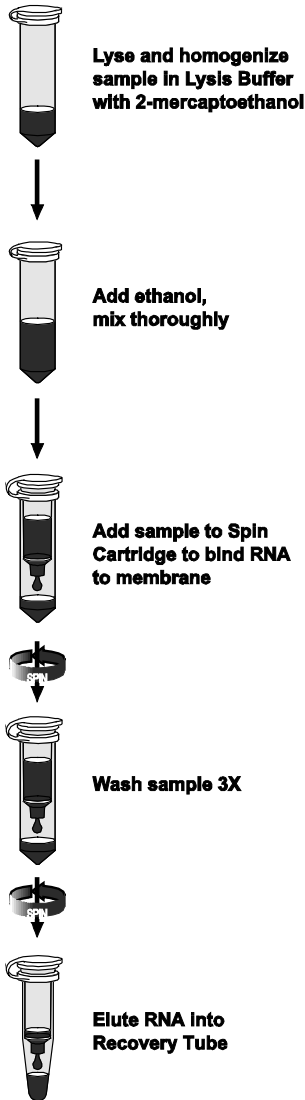
Starting Material:	Varies
Cartridge Binding Capacity:	~ 1 mg nucleic acid
Cartridge Reservoir Capacity:	700 μ L
Wash Tube Capacity:	2.0 mL
Centrifuge Compatibility:	Capable of $>12,000 \times g$
Elution Volume:	30 μ L–3 \times 100 μ L (see Parameters , 13)
RNA Yield:	Varies with sample type and quality (see page 59)

Continued on next page

System Description, Continued

Workflow

The flow chart below illustrates the steps for isolating total RNA using the PureLink® RNA Mini Kit.



Methods

General Guidelines

Introduction

Review the information in this section **before** beginning. Guidelines are provided in this section for handling RNA and sample collection.

Guidelines for Handling RNA

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

- Use sterile, disposable, and individually wrapped plasticware.
 - 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 gloves 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 (page 67) to remove RNase contamination from work surfaces and non-disposable items such as centrifuges and pipettes that will be used during purification.
-

Storage of Purified RNA

Store your purified RNA on ice when using the RNA within a few hours of isolation. For long-term storage, store your purified RNA at -80°C .

Continued on next page

General Guidelines, Continued

Guidelines for Sample Collection

When collecting your samples, follow the guidelines below to minimize RNA degradation and to maximize RNA yield.

- Always wear disposable gloves while handling samples and reagents to prevent RNase contamination.
- Work quickly during sample harvesting and use RNase-free dissection tools and containers (scalpels, dishes, tubes etc.).
- Use RNase AWAY[®] Reagent (page 67) to remove RNase contamination from work surfaces.
- When purifying total RNA from fresh samples, keep fresh cell and tissue samples on ice immediately after harvesting; quickly proceed to sample **Lysis and Homogenization**.
- When purifying total RNA from frozen samples, freeze samples immediately after collection in liquid nitrogen or on dry ice. Keep frozen samples at -80°C or in liquid nitrogen until proceeding to sample **Lysis and Homogenization**.

Whole blood: We recommend collecting whole blood in the presence of anticoagulants (e.g. EDTA or citrate) and storing at 4°C until use. Freshly drawn blood can be used without anticoagulants. You may also process frozen blood.



- Both Lysis Buffer and Wash Buffer I contain guanidine isothiocyanate (an irritant). This chemical is harmful when in contact with the skin, or when it is inhaled or ingested.
- **Do not** add bleach or acidic solutions directly to solutions or sample preparation waste that contains guanidinium isothiocyanate, as reactive compounds and toxic gases are formed.
- Solutions containing ethanol are considered flammable. Use appropriate precautions when using this chemical.

For your protection, always wear a laboratory coat, gloves and safety glasses when handling these chemicals. Dispose of the buffers and chemicals in appropriate waste containers.

Continued on next page

General Guidelines, Continued

TRIzol[®] Reagent

To isolate RNA from samples that are difficult to lyse (*e.g.*, fibrous animal or plant tissues), or to purify ultrapure total RNA for sensitive downstream applications, you can use TRIzol[®] Reagent (page 67) followed by purification using the PureLink[®] RNA Mini Kit (see page 49 for details).

DNase Treatment of RNA

On-column PureLink[®] DNase treatment during RNA purification

If your downstream application requires DNA-free total RNA, you can use the convenient on-column PureLink[®] DNase treatment *during* your purification procedure. Each sample-specific protocol refers you to **On-column PureLink[®] DNase Treatment Protocol** (page 63) at the appropriate step in the procedure.

The on-column PureLink[®] DNase treatment eliminates the need for DNase treatment and clean-up after purification (see page 65 for more details).

DNase I treatment after RNA purification

You may also perform a DNase I, Amplification Grade (page 67) digestion of the RNA sample *after* purification (page 65). This may, however, result in reduced RNA yield.

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 microcentrifuge pestle:

1. Cool the microcentrifuge tube on ice.
 2. Transfer the tissue sample into the microcentrifuge tube.
 3. Add Lysis Buffer and use up-and-down with twisting movements to disrupt the sample between the tube wall and the pestle.
 4. After lysis, homogenize the sample as specified in your sample-specific protocol.
-

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General Guidelines, Continued

Mortar and Pestle

RNase-free mortars and pestles are used in combination with liquid nitrogen to disrupt and lyse frozen and fibrous tissue samples.

To use the mortar and pestle:

1. Place your tissue sample and a small amount of liquid nitrogen into the mortar and grind the tissue into a powder using the pestle.
 2. Transfer the frozen tissue powder into a liquid nitrogen-cooled tube of appropriate size and allow the liquid nitrogen to evaporate.
 3. Add Lysis Buffer to the powdered tissue as directed in your sample-specific protocol.
Important: Do not let the tissue sample thaw before you add the Lysis Buffer.
 4. After lysis, homogenize the sample as specified in your sample-specific protocol.
-

Homogenizer

The Homogenizer (page 67) is designed to homogenize cell or tissue lysates via centrifugation, prior to nucleic acid purification. The Homogenizer consists of a cartridge with a specialized membrane that fits inside the Collection Tube that contains the lysate. The Collection Tube is placed into 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. The Homogenizer 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 67).

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General Guidelines, Continued

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 the rotor-stator:

1. Transfer your sample into a round-bottomed tube of appropriate size and add the appropriate volume of Lysis Buffer (Refer to your sample-specific protocol to determine the amount of Lysis Buffer needed).

Note: When using a rotor-stator homogenizer, you may need to use a greater volume of Lysis Buffer than is provided in the PureLink™ RNA Mini kit. For these instances, you can purchase our bulk PureLink™ 96 RNA Lysis Buffer (page 67) to provide you with the extra buffer needed.

2. Insert the rotor-stator probe tip into the sample and homogenize for 5–90 seconds, depending on the toughness of sample.

Note: Avoid foaming of your sample by keeping the tip of the probe submerged in the lysis solution while holding the tip against the tube wall. Refer to the manual provided with your rotor-stator for more information. Rotor-stators are available in various sizes. Common models include ULTRA-TURRAX® (IKA Works, Inc.) and Polytron® Homogenizer (Kinematica, Brinkmann Instruments).

General Guidelines, Continued

Sample Lysis and Homogenization

Use the tables below and on the next page to determine the best method for lysing and homogenizing your specific sample type.

Note: Be careful to not exceed the maximum binding capacity of the cartridge (~1 mg nucleic acid) when selecting the amount of starting material, as this will decrease the total RNA yield. See page 59.

Sample Type	Lysis Options	Homogenization Options	Comments
Animal and Plant Cells	Lysis Buffer, vortexing	<ul style="list-style-type: none"> • 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)	<ul style="list-style-type: none"> • Homogenizer • Syringe and needle 	--
	Mortar and pestle in liquid nitrogen (10-100 mg tissue)	<ul style="list-style-type: none"> • 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)	<ul style="list-style-type: none"> • 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.

Continued on next page

General Guidelines, Continued

Sample Lysis and Homogenization, continued

Sample Type	Lysis Options	Homogenization Options	Comments
Plant Tissue: Frozen or Fresh Fibrous	Mortar and pestle in liquid nitrogen	<ul style="list-style-type: none"> • Homogenizer • Rotor-stator 	We recommend using a mortar and pestle with liquid nitrogen for more complete lysis than a rotor-stator alone.
Plant Tissue: Fresh, Soft	Rotor-stator		Rotor-stator lyses and homogenizes simultaneously and can be used with all tissue amounts up to 200 mg.
Fresh Whole Blood	Lysis Buffer, vortexing		--
Yeast Cells	Enzyme digestion by Zymolase, followed by Lysis Buffer, vortexing		Not recommended for kinetic experiments.
	Mortar and pestle with crushed dry ice	<ul style="list-style-type: none"> • Homogenizer • Syringe and needle • Rotor-stator 	Recommended for kinetic experiments.
Bacteria	Digestion with lysozyme, vortexing	<ul style="list-style-type: none"> • Homogenizer • Syringe and needle • Rotor-stator 	--
Liquid samples	Lysis Buffer, vortexing		--

Buffer Preparation and Parameters

Preparing Wash Buffer II with Ethanol

Before using Wash Buffer II for the first time:

1. Add 60 mL (for Cat. no. 12183018A) or 300 mL (for Cat. no. 12183025) of 96–100% ethanol directly to the bottle.
 2. Check the box on the Wash Buffer II label to indicate that ethanol was added.
 3. Store Wash Buffer II with ethanol at room temperature.
-

Preparing Lysis Buffer with 2-Mercaptoethanol

Prepare a fresh amount of Lysis Buffer containing 1% 2-mercaptoethanol for each purification procedure. Add 10 μ L 2-mercaptoethanol for each 1 mL Lysis Buffer.

Use the tables provided with each sample-specific protocol to determine the correct volume of Lysis Buffer with 2-mercaptoethanol required for your sample lysis.



Note

Dithiothreitol (DTT) can be used as an alternative reducing agent in place of 2-mercaptoethanol in the Lysis Buffer.

Prepare a fresh amount of Lysis Buffer containing 40 mM DTT for each purification procedure. Add 20 μ L of 2 M DTT for each 1 mL of L3 Lysis Buffer.

Prepare fresh DTT solution, by resuspending 308.5 mg DTT (Cat. no. 15508-013) in 1 mL of RNase-free water.

Continued on next page

Buffer Preparation and Parameters, Continued

Amount of Lysis Buffer Needed

The amount of Lysis Buffer needed is determined by both the amount of your starting material as well as your sample type. Each sample-specific protocol provides a table indicating the recommended amount of Lysis Buffer with 2-mercaptoethanol needed for your sample type and starting amount.

If your sample contains more than an average amount of RNA, or if you are using a rotor-stator homogenizer, you may need to use a greater volume of Lysis Buffer than is provided in the PureLink™ RNA Mini kit. For these larger samples you can purchase our bulk PureLink™ 96 RNA Lysis Buffer (page 67) to provide you with the extra buffer needed to complete your sample lysis. Refer to your sample-specific protocol for the correct volume of Lysis Buffer to use.

Buffer Preparation and Parameters, Continued

Elution Parameters

Elution Reagent

RNA can be eluted from the 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 to elute your RNA.

Elution Volume

RNA yield is dependent on sample type, size, and quality. Depending on your expected RNA yield, and your sample source and starting amount, use between 30 μL –3 \times 100 μL RNase Free Water (or Tris-Buffer) for each elution. Example yields for various sample types and amounts are provided on page 59.

General Recommendations for Elution Volume

For expected RNA yields of 100 μg or less, perform one elution using 30–100 μL RNase-Free Water or Tris buffer. For large samples and for expected RNA yields >100 μg , perform sequential 100 μL elutions on your sample using RNase-Free Water or Tris buffer.

For example, to obtain RNA yields of 100–500 μg , perform 2 sequential elutions of 100 μL per elution (2 \times 100 μL) and for RNA yields of 500–1,000 μg , perform 3 sequential elutions of 100 μL per elution (3 \times 100 μL). Use the table below to help determine the correct volume of elution reagent to use and refer to your sample-specific protocol for more details.

Elution Volume	RNA Yield Quantity
30–100 μL	\leq 100 μg
2 \times 100 μL	100–500 μg
3 \times 100 μL	500–1,000 μg

Purifying RNA from Animal and Plant Cells

Introduction

This section provides instructions for purifying total RNA from animal and plant cells. Separate protocols are provided for $\leq 5 \times 10^6$ cells (suspension and monolayer) and for 5×10^6 – 5×10^7 cells.

Materials Needed

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

- 2-mercaptoethanol
- 70% ethanol (in RNase-Free Water)
- Microcentrifuge capable of centrifuging 12,000 \times g
- 1.5 mL RNase-free microcentrifuge tubes
- 15 mL RNase-free tubes ($>10^7$ cells per sample)
- PBS ($>10^7$ cells per sample)
- RNase-free pipette tips
- *Optional:* PureLink[®] DNase (page 67)

For $\leq 5 \times 10^6$ cells:

- Homogenizer (see page 67 and page 7) **or**,
- RNase-free syringe (1 mL) with 18-21 gauge needle **or**,
- Rotor-stator homogenizer (page 8)

For 5×10^6 – 5×10^7 cells:

Rotor-stator homogenizer (page 8)

Amount of Lysis Buffer Needed

Before beginning the lysis and homogenization steps, prepare a fresh amount of Lysis Buffer containing 1% 2-mercaptoethanol for each purification procedure. Add 10 μ L 2-mercaptoethanol for each 1 mL Lysis Buffer.

Using the table below, determine the correct amount of Lysis Buffer needed for your sample type and amount.

Note: For larger than average samples, or if using a rotor-stator, additional Lysis Buffer may be required. See page 12 for details.

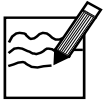
Number of cells in your sample	Amount of Lysis Buffer Needed (prepared with 2-mercaptoethanol)
$\leq 1 \times 10^6$	0.3 mL*
1×10^6 – 5×10^6	0.6 mL
5×10^6 – 5×10^7	0.6 mL per 5×10^6 cells For example: use 1.2 mL for 1×10^7 cells and 6.0 mL for 5×10^7 cells

*Use 0.6 mL if using rotor-stator for lysis or homogenization.

Continued on next page

Purifying RNA from Animal and Plant Cells,

Continued



Note

For samples that are difficult to lyse, you can use TRIzol® Reagent followed by purification using the PureLink® RNA Mini Kit, (see page 49).

Lysis and Homogenization: $\leq 5 \times 10^6$ Suspension Cells

Follow the steps below to prepare lysates from $\leq 5 \times 10^6$ suspension cells:

1. Transfer cells to an appropriately sized RNase-free tube and centrifuge at $2,000 \times g$ for 5 minutes at 4°C to pellet. Discard the growth medium from the tube.
2. Using RNase-free pipette tips, add the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol to your sample (refer to previous page for correct amounts).
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, you may skip this step.

4. Proceed with **one** of the following homogenization options at room temperature:
 - Transfer the lysate to a Homogenizer (page 67) inserted in a Collection Tube and centrifuge at $12,000 \times g$ for 2 minutes. Remove the Homogenizer when done, **or**
 - Pass the lysate 5–10 times through an 18–21-gauge needle attached to an RNase-free syringe, **or**
 - Transfer the lysate to an appropriately sized RNase-free tube and homogenize using a rotor-stator homogenizer at maximum speed for at least 45 seconds. Centrifuge the homogenate at $\sim 2,600 \times g$ for 5 minutes, then transfer the supernatant to a clean RNase-free tube.

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

Continued on next page

Purifying RNA from Animal and Plant Cells, Continued

Lysis and Homogenization: $\leq 5 \times 10^6$ Monolayer Cells

Follow the steps below to prepare lysates from $\leq 5 \times 10^6$ monolayer cells:

1. Remove the growth medium from the cells.
2. Using RNase-free pipette tips, add the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol to your sample (refer to page 14 for correct amounts).
3. Proceed with **one** of the following homogenization options at room temperature:
 - Transfer the lysate to a Homogenizer (page 67) inserted in a Collection Tube and centrifuge at $12,000 \times g$ for 2 minutes. Remove the Homogenizer when done, **or**
 - Transfer the lysate to a 1.5 mL RNase-free tube and pass 5–10 times through an 18-21-gauge needle attached to an RNase-free syringe, **or**
 - Transfer the lysate to an appropriately sized RNase-free tube and homogenize using a rotor-stator homogenizer at maximum speed for at least 45 seconds. Centrifuge the homogenate at $\sim 2,600 \times g$ for 5 minutes, then transfer the supernatant to a clean RNase-free tube.

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

Continued on next page

Purifying RNA from Animal and Plant Cells, Continued

Lysis and Homogenization: 5×10^6 – 5×10^7 Suspension Cells

Follow the steps below to prepare lysates from 5×10^6 – 5×10^7 cells.

Note: This protocol uses a rotor-stator homogenizer. Use 15 mL RNase-free tubes to compensate for volume expansion that occurs during homogenization using a rotor-stator.

1. Transfer cells to a 15 mL RNase-free tube and centrifuge at $2,000 \times g$ for 5 minutes at 4°C to pellet. Remove and discard the supernatant.
2. Using RNase-free pipette tips, add the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol to your sample (refer to page 14 for correct amounts).
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 $\sim 2,600 \times g$ for 5 minutes at room temperature.
6. Transfer the supernatant to a clean 15-mL RNase-free tube.

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

Continued on next page

Purifying RNA from Animal and Plant Cells, Continued

Lysis and Homogenization: Frozen Cell Pellets

Follow the steps below to prepare lysates from frozen cell pellets. For 5×10^6 – 5×10^7 cells, we recommend homogenizing with a rotor-stator homogenizer.

1. Transfer the frozen cell pellet to an appropriately sized RNase-free tube.
2. Using RNase-free pipette tips, add the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol to your sample (refer to page 14 for correct amounts).
Note: If you are using a rotor-stator homogenizer, you may skip ahead to Step 4.
3. Vortex at high speed until the cell pellet is completely dispersed and the cells appear lysed.
4. Proceed with **one** of the following homogenization options at room temperature:
 - Transfer the lysate to a Homogenizer (page 67) inserted in an RNase-free tube and centrifuge at $12,000 \times g$ for 2 minutes. Remove the Homogenizer cartridge when done, **or**
 - Pass the lysate 5–10 times through an 18–21-gauge needle attached to an RNase-free syringe, **or**
 - Transfer the lysate to an appropriately sized RNase-free tube and homogenize using a rotor-stator homogenizer at maximum speed for at least 45 seconds. Centrifuge the homogenate at $\sim 2,600 \times g$ for 5 minutes, then transfer supernatant to a clean RNase-free tube.

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

Continued on next page

Purifying RNA from Animal and Plant Cells, Continued

Binding, Washing, and Elution

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

1. Add one volume 70% ethanol to each volume of cell homogenate (prepared as described in the sample-specific protocols (pages 15–18).
Note: If part of the sample was lost during homogenization, adjust the volume of ethanol accordingly.
2. Vortex to mix thoroughly and to 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 Spin Cartridge (with the Collection Tube).
4. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. Discard the flow-through, and reinsert the Spin Cartridge into the same Collection 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 Spin Cartridge.
5. **Repeat** Steps 3–4 until the entire sample is processed.
Optional: If DNA-free total RNA is required, proceed to **On-column PureLink® DNase Treatment Protocol** (page 63).
6. Add 700 μL Wash Buffer I to the Spin Cartridge. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. Discard the flow-through **and** the Collection Tube. Place the Spin Cartridge into a **new** Collection Tube.
7. Add 500 μL Wash Buffer II with ethanol (page 11) to the Spin Cartridge.
8. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. Discard the flow-through and reinsert the Spin Cartridge into the same Collection Tube.

Continued on next page

Purifying RNA from Animal and Plant Cells, Continued

Binding, Washing, and Elution, continued

9. Repeat Steps 7–8 **once**.
 10. Centrifuge the Spin Cartridge at $12,000 \times g$ for 1-2 minutes to dry the membrane with attached the RNA. **Discard** the Collection Tube and insert the Spin Cartridge into a Recovery Tube.
 11. Add 30 μL – $3 \times 100 \mu\text{L}$ RNase-Free Water to the center of the Spin Cartridge (see **Elution Parameters**, page 13).
 12. Incubate at room temperature for 1 minute.
 13. Centrifuge the Spin Cartridge for 2 minutes at $\geq 12,000 \times g$ at room temperature to elute the RNA from the membrane into the Recovery tube.
Note: If you are performing sequential elutions, collect all elutes into the same tube (see page 13 for **Elution Parameters**).
 14. Store your purified RNA or proceed to **Analyzing RNA Yield and Quality** (page 53) or to **DNase I Treatment After RNA Purification** (page 65).
-

Purifying RNA from Animal Tissues

Introduction

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



Note

- Protocols for frozen or fibrous fresh tissue begin on page 23. Protocols for soft fresh tissue begin on page 25.
- For samples that are difficult to lyse, or to purify ultrapure total RNA for downstream applications, you can use TRIzol® Reagent followed by purification using the PureLink® RNA Mini Kit, (page 49).



Important

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

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	<ul style="list-style-type: none">• Microcentrifuge Pestle• Rotor-stator
	10–200 mg	<ul style="list-style-type: none">• Mortar and pestle with liquid nitrogen• Rotor-stator
Fresh soft	≤ 100 mg	<ul style="list-style-type: none">• Microcentrifuge Pestle• Rotor-stator
	100–200 mg	<ul style="list-style-type: none">• Rotor-stator

Continued on next page

Purifying RNA from Animal Tissues, Continued

Materials Needed

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

- 2-mercaptoethanol
 - 70% ethanol (in RNase-Free Water)
 - 96–100% ethanol
 - *Optional:* PureLink® DNase (page 67)
 - **One** of the following for tissue disruption and lysis:
 - RNase-free glass, Teflon, or plastic pestle (for ≤ 100 mg tissue) *or*
 - Rotor-stator homogenizer (required for ≥ 100 mg of tissue; see page 8)
 - **One** of the following for homogenization:
 - 18–20-gauge needle with RNase-free syringe *or*
 - Homogenizer (page 67 and page 7) *or*
 - Rotor-stator homogenizer (page 8)
 - RNase-free tubes (1.5 mL–15 mL, depending on amount of tissue, refer to sample-specific protocols for details)
 - Liquid nitrogen (for lysis of ≥ 10 mg of frozen tissue or fresh fibrous tissue)
 - Microcentrifuge capable of centrifuging $12,000 \times g$
 - RNase-free pipette tips
-

Amount of Lysis Buffer Needed

Before beginning the lysis and homogenization steps, prepare a fresh amount of Lysis Buffer containing 1% 2-mercaptoethanol for each purification procedure. Add 10 μ L 2-mercaptoethanol for each 1 mL Lysis Buffer.

Using the table below, determine the correct amount of Lysis Buffer needed for your sample type and amount.

Note: For larger than average samples, or if using a rotor-stator, additional Lysis Buffer may be required. See page 12 for details.

Amount of tissue	Lysis Buffer (prepared with 2-mercaptoethanol)
≤ 10 mg	0.3 mL*
10–30 mg	0.6 mL
30–200 mg	0.6 mL per 30 mg of tissue

*Use 0.6 mL if using rotor-stator for lysis or homogenization.

Continued on next page

Purifying RNA from Animal Tissues, Continued

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

Use **one** of the following protocols (microcentrifuge pestle, or rotor-stator) to prepare ≤10 mg of frozen or fresh fibrous tissue.

Microcentrifuge Pestle Protocol

1. Cool a 1.5 mL RNase-free microcentrifuge tube on ice and transfer your tissue sample into the tube.
2. Using RNase-free pipette tips, add the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol to your sample (refer to previous page for correct amounts).
3. Mince tissue using an RNase-free pestle with up/down and twisting movements in the tube until tissue is thoroughly disrupted and lysed.
4. Proceed with **one** of the following homogenization options at room temperature:
 - Transfer the lysate to a Homogenizer (page 67) inserted in an RNase-free tube and centrifuge at $12,000 \times g$ for 2 minutes. Remove the Homogenizer when done, **or**
 - Pass the lysate 5–10 times through an 18–21-gauge needle attached to an RNase-free syringe, then centrifuge at $12,000 \times g$ for 2 minutes. Transfer the supernatant to a new RNase-free tube.

Proceed to **Binding, Washing, and Elution** (page 27).

Rotor-Stator Protocol:

1. Place a 4 mL round-bottom RNase-free tube on ice. Transfer your tissue sample into the tube.
2. Using RNase-free pipette tips, add the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol to your sample (refer to previous page for correct amounts).
3. Homogenize sample for 30–40 seconds.
4. Centrifuge at $\sim 2,600 \times g$ for 5 minutes at room temperature.
5. Carefully transfer the supernatant to a new RNase-free tube.

Proceed to **Binding, Washing, and Elution** (page 27).

Continued on next page

Purifying RNA from Animal Tissues, Continued

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

Use **one** of the following protocols (mortar and pestle or rotor-stator) to prepare 10–100 mg of frozen or fresh fibrous tissue.

Mortar and Pestle Protocol:

1. Place your tissue sample in an RNase-free mortar and add liquid nitrogen into the mortar. Grind the tissue thoroughly to a powder using an RNase-free pestle.
2. Cool an RNase-free 2 mL round-bottom microcentrifuge tube on liquid nitrogen, then transfer the tissue powder to the tube. Allow the liquid nitrogen to evaporate.
3. Using RNase-free pipette tips, add the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol to your sample (refer to page 22 for correct amounts).
4. Proceed with **one** of the following homogenization options at room temperature:
 - Transfer up to 0.6 mL of the lysate to a Homogenizer (page 67) that is inserted into an RNase-free tube. Centrifuge at $12,000 \times g$ for 2 minutes. Remove the Homogenizer from the tube when done, **or**
 - Pass the lysate from through an 18–21-gauge needle attached to an RNase-free syringe 5–10 times, then centrifuge at $12,000 \times g$ for 2 minutes. Transfer the supernatant to a new RNase-free tube.

Proceed to **Binding, Washing, and Elution** (page 27).

Rotor-stator Protocol:

1. Place a 4 mL RNase-free round-bottomed tube on ice, and transfer your tissue sample into the tube.
2. Using RNase-free pipette tips, add the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol to your sample (refer to page 22 for correct amounts).
3. Homogenize sample for 30–40 seconds using a rotor-stator.
4. Centrifuge at $\sim 2,600 \times g$ for 5 minutes at room temperature.
5. Carefully transfer the supernatant to a new RNase-free tube.

Proceed to **Binding, Washing, and Elution** (page 27).

Continued on next page

Purifying RNA from Animal Tissues, Continued

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.

Note: This protocol uses a rotor-stator homogenizer.

1. Place a 15 mL RNase-free round-bottomed tube on ice and transfer your tissue sample into the tube.
2. Using RNase-free pipette tips, add the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol to your sample (refer to page 22 for correct amounts).
3. Homogenize sample for at least 45 seconds using a rotor-stator at maximum speed.
4. Centrifuge at $\sim 2,600 \times g$ for 5 minutes at room temperature.
5. Carefully transfer the supernatant to a new RNase-free tube.

Proceed to **Binding, Washing, and Elution** (page 27).

Lysis and Homogenization: ≤ 100 mg Fresh Soft Tissue

Use **one** of the following protocols (microcentrifuge pestle, or rotor-stator) to prepare up to 100 mg of fresh soft tissue.

Microcentrifuge Pestle Protocol:

1. Transfer your tissue sample to a 1.5 mL or 2.0 mL RNase-free round-bottomed tube.
 2. Using RNase-free pipette tips, add the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol to your sample (refer to page 22 for correct amounts).
 3. 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.
 4. Centrifuge at $12,000 \times g$ for 2 minutes at room temperature. Transfer supernatant to a clean RNase-free tube.
-

Continued on next page

Purifying RNA from Animal Tissues, Continued

Lysis and Homogenization: ≤100 mg Fresh Soft Tissue, continued

5. Proceed with **one** of the following homogenization options at room temperature:
 - Insert a Homogenizer (page 67) into an RNase-free tube and transfer the lysate from Centrifuge at $12,000 \times g$ for 2 minutes. Remove the Homogenizer when done, **or**
 - Pass the lysate 5–10 times through an 18–21-gauge needle attached to an RNase-free syringe, then centrifuge at $12,000 \times g$ for 2 minutes. Transfer the supernatant to a new RNase-free tube.

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

Rotor-Stator Protocol:

1. Transfer tissue to a 4 mL round-bottomed tube.
2. Using RNase-free pipette tips, add the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol to your sample (refer to page 22 for correct amounts).
3. Quickly homogenize the sample using a rotor-stator at maximum speed for at least 45 seconds.
4. Centrifuge at $\sim 2,600 \times g$ for 5 minutes at room temperature.
5. Transfer the supernatant to a clean RNase-free tube.

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

Lysis and Homogenization: 100–200 mg Fresh Soft Tissue

Use the following protocol to prepare 100–200 mg of soft fresh tissue with a rotor-stator homogenizer.

1. Transfer tissue to a 15-mL round-bottomed tube.
2. Using RNase-free pipette tips, add the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol to your sample size (refer to page 22 for correct amounts).
3. Quickly homogenize the sample using a rotor-stator at maximum speed for at least 45 seconds.
4. Centrifuge at $\sim 2,600 \times g$ for 5 minutes at room temperature.
5. Transfer the supernatant to a clean RNase-free tube.

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

Continued on next page

Purifying RNA from Animal Tissues, Continued

Binding, Washing, and Elution

1. Add one volume of 70% ethanol to the tissue homogenate (see previous page).
2. Mix thoroughly by shaking or vortexing to disperse any visible precipitate that may form after adding ethanol.
3. Transfer ≤ 700 μL of the sample (including any remaining precipitate) to the Spin Cartridge.
4. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. Discard the flow-through, and reinsert the Spin Cartridge in the same Collection Tube.
5. **Repeat** Steps 3–4 until the entire sample is processed.
Optional: If DNA-free total RNA is required, proceed to **On-column PureLink® DNase Treatment Protocol** (page 63).
6. Add 700 μL Wash Buffer I to the Spin Cartridge. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. Discard the flow-through **and** the Collection Tube. Place the Spin Cartridge into a **new** Collection Tube.
7. Add 500 μL Wash Buffer II with ethanol (page 11) to the Spin Cartridge.
8. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. Discard the flow-through, and reinsert the Spin Cartridge in the same Collection Tube.
9. Repeat Steps 7–8 **once**.
10. Centrifuge the Spin Cartridge at $12,000 \times g$ for 1 minute at room temperature to dry the membrane with attached RNA. **Discard** the Collection Tube and insert the Spin Cartridge into a Recovery Tube.
11. Add 30 μL –100 μL RNase-Free Water to the center of the Spin Cartridge (see **Elution Parameters**, page 13).
12. Incubate at room temperature for 1 minute.
13. Centrifuge for 2 minutes at $\geq 12,000 \times g$ at room temperature.
14. Store your purified RNA (see page 4), or proceed to **Analyzing RNA Yield and Quality** (page 53), or to **DNase I Treatment After RNA Purification** (page 65).

Continued on next page

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 or fresh fibrous tissue begin on page 30. Protocols for soft fresh tissue begin on page 31.
 - For samples that are difficult to lyse, or to purify ultrapure total RNA for downstream applications, you can use TRIzol® Reagent followed by purification with the PureLink® RNA Mini Kit (page 49).
-



Important

- Frozen tissue must remain frozen at -80°C prior to lysis. Cool tubes on dry ice before placing frozen tissue in them. **Thawing of frozen tissue prior to lysis may result in RNA degradation and reduced RNA yield.**
 - For plant tissues rich in polyphenolics or starch (*e.g.*, pine needles, potato tubers), we recommend using Plant RNA Reagent for isolation of RNA (see page 67).
 - Fast and complete disruption of tissue during the lysis step is important to prevent RNA degradation.
-

Continued on next page

Purifying RNA from Plant Tissues, Continued

Materials Needed

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

- 2-mercaptoethanol
 - 96–100% ethanol
 - RNase-free pipette tips
 - 1.5–15 mL round-bottomed RNase-free tubes (depending on amount of tissue)
 - Microcentrifuge capable of centrifuging 12,000 × g
 - *Optional:* PureLink™ DNase (page 67)
 - **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 7)
-

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	Mortar and pestle with liquid nitrogen
Fresh soft	Rotor-stator

Amount of Lysis Buffer Needed

Before beginning the lysis and homogenization steps, prepare a **fresh** amount of Lysis Buffer containing 1% 2-mercaptoethanol for each purification procedure. Add 10 µL 2-mercaptoethanol for each 1 mL Lysis Buffer.

Using the table below, determine the correct amount of Lysis Buffer needed for your sample type and amount.

Note: If using a rotor-stator, additional Lysis Buffer may be required. See page 12 for details.

Amount of tissue	Lysis Buffer (prepared with 2-mercaptoethanol)
≤100 mg	0.5 mL*
100–200 mg	1.0 mL
200–250 mg	1.5 mL

*Use 0.6 mL if using rotor-stator for lysis or homogenization.

Continued on next page

Purifying RNA from Plant Tissues, Continued

Lysis and Homogenization: Frozen or Fresh Fibrous Tissue

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

Fresh fibrous tissue only: Before beginning, on ice, quickly cut tissue into small, ≤ 0.5 cm² pieces using an RNase-free scalpel and tweezers.

1. Add liquid nitrogen to an RNase-free mortar and grind frozen or fresh fibrous tissue thoroughly using an RNase-free pestle.
2. Transfer the tissue powder to an RNase-free, appropriately sized round-bottom microcentrifuge tube that has been cooled on liquid nitrogen. Allow liquid nitrogen to evaporate.
3. Immediately add the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol to your sample (refer to previous page for correct amounts).
4. Proceed with **one** of the following homogenization options at room temperature:
 - a. Vortex the lysate from to disperse the sample. Incubate for 3 minutes at room temperature.
 - b. Transfer up to 0.5 mL of the lysate to a Homogenizer (page 67) inserted in an RNase-free tube. Centrifuge at $12,000 \times g$ for 2 minutes. Remove the Homogenizer when done.
Note: Use additional homogenizers for each 0.5 mL sample.

or

- a. Homogenize your sample for at least 45 seconds using a rotor-stator at maximum speed.
- b. Centrifuge at $\sim 2,600 \times g$ for 5 minutes at room temperature.
- c. Carefully transfer the supernatant to a **new** RNase-free tube.

Proceed to **Binding, Washing, and Elution** (page 32).

Continued on next page

Purifying RNA from Plant Tissues, Continued

Lysis and Homogenization: Fresh Soft Tissue

Use the following protocol to prepare soft fresh tissue.

Note: This protocol uses a rotor-stator.

1. On ice, quickly cut tissue into small, ≤ 0.5 cm² pieces using an RNase-free scalpel and tweezers. Transfer tissue to an appropriately sized round-bottomed microcentrifuge tube.
2. Immediately add the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol to your sample (refer to page 29 for correct amounts).
3. Quickly homogenize the sample using a rotor-stator at maximum speed for at least 45 seconds.
4. Centrifuge at $\sim 2,600 \times g$ for 5 minutes at room temperature.
5. Transfer the supernatant to a **new** RNase-free tube.

Proceed to **Binding, Washing, and Elution** (page 32).

Continued on next page

Purifying RNA from Plant Tissues, Continued

Binding, Washing, and Elution

Follow the steps below to bind, wash, and elute the RNA from your plant tissue sample.

1. Add 0.5 volume 96–100% ethanol to each volume of tissue homogenate.
2. Mix thoroughly by shaking or vortexing to disperse any visible precipitate that may form after adding ethanol.
3. Transfer up to 700 μL of your sample (including any remaining precipitate) to the Spin Cartridge (with a Collection Tube).
4. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. Discard the flow-through, and reinsert the Spin Cartridge in the same Collection Tube.
5. **Repeat** Steps 3–4 until the entire sample is processed.
Optional: If DNA-free total RNA is required, proceed to **On-column PureLink™ DNase Treatment Protocol** (page 63).
6. Add 700 μL Wash Buffer I to the Spin Cartridge. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. Discard the flow-through **and** the Collection Tube and place the Spin Cartridge into a **new** Collection Tube.
7. Add 500 μL Wash Buffer II with ethanol (page 11) to the Spin Cartridge.
8. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. Discard the flow-through and reinsert the Spin Cartridge in the same Collection Tube.
9. Repeat Steps 7–8 **once**.

Continued on next page

Purifying RNA from Plant Tissues, Continued

Binding, Washing, and Elution, continued

10. Centrifuge the Spin Cartridge with Collection tube at $12,000 \times g$ for 1 minute at room temperature to dry the membrane with attached RNA. Discard the Collection Tube and insert the Spin Cartridge into a Recovery Tube.
 11. Add $30 \mu\text{L}$ – $3 \times 100 \mu\text{L}$ RNase-Free Water to the center of the Spin Cartridge, (see **Elution Parameters**, page 13).
 12. Incubate at room temperature for 1 minute.
 13. Centrifuge the Spin Cartridge for 2 minutes at $\geq 12,000 \times g$ at room temperature.
Note: If you are performing sequential elutions, collect all elutes into the same tube.
 14. Store your purified RNA (see page 4), or proceed to **Analyzing RNA Yield and Quality** (page 53) or to **DNase I Treatment After RNA Purification** (page 65).
-

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 4°C until use. Freshly drawn blood can be used without anticoagulants. You may also process frozen blood, however, the yield and quality of RNA may be lower.

Materials Needed

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

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

Preparing Lysis Buffer for Whole Blood Samples

Before beginning, prepare the Lysis Buffer containing 1% 2-mercaptoethanol **fresh** for each.

To prepare enough solution to purify ≤0.2 mL of whole blood, add 2 μL 2-mercaptoethanol to 0.2 mL Lysis Buffer.

Continued on next page

Purifying RNA from Whole Blood, Continued

Isolating RNA from Whole Blood

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

1. Place ≤ 0.2 mL of whole blood sample to a 1.5 mL RNase-free microcentrifuge tube.
2. Add 0.2 mL Lysis Buffer prepared with 2-mercaptoethanol (see previous page).
3. Vortex thoroughly to disrupt and lyse the blood cells, then centrifuge the lysate at $12,000 \times g$ for 2 minutes at room temperature.
4. Transfer the supernatant to a clean 1.5 mL RNase-free microcentrifuge tube.
5. Add 200 μ L 100% ethanol to the microcentrifuge tube. Disperse any precipitate by vortexing or pipetting up and down several times (use RNase-free pipette tips).
6. Transfer the sample (including any remaining precipitate) to the Spin Cartridge (with a Collection Tube). Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. Discard the flow-through.

Optional: If DNA-free total RNA is required, proceed to **On-column PureLink® DNase Treatment Protocol** (page 63).

7. Add 700 μ L Wash Buffer I to the Spin Cartridge. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. Discard the flow-through **and** the Collection Tube. Place the Spin Cartridge into a **new** Collection Tube.
8. Add 500 μ L Wash Buffer II with ethanol (page 11) to the Spin Cartridge.
9. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. Discard the flow-through, and reinsert the Spin Cartridge in the same Collection Tube.
10. Repeat Steps 8–9 **once**.

Continued on next page

Purifying RNA from Whole Blood, Continued

Isolating RNA from Whole Blood, continued

11. Centrifuge the Spin Cartridge at $12,000 \times g$ for 1 minute at room temperature to dry the membrane with attached RNA. Discard the Collection Tube and insert the Spin Cartridge into a Recovery Tube.
 12. Add $30 \mu\text{L}$ – $3 \times 100 \mu\text{L}$ RNase-Free Water to the center of the Spin Cartridge, (see **Elution Parameters**, page 13).
 13. Incubate at room temperature for 1 minute.
 14. Centrifuge the Spin Cartridge and Recovery Tube for 2 minutes at $\geq 12,000 \times g$ at room temperature.
Note: If you are performing sequential elutions, collect all elutes into the same tube.
 15. Store your purified RNA (see page 4), or proceed to **Analyzing RNA Yield and Quality** (page 53) or to **DNase I Treatment After RNA Purification** (page 65).
-

Purifying RNA from Yeast Cells

Introduction

This section provides instructions for preparing total RNA for 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 and for optimal yields of total RNA.
-

Materials Needed

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

- 2-mercaptoethanol
- Microcentrifuge capable of centrifuging $12,000 \times g$
- 1.5 mL RNase-free microcentrifuge tubes
- RNase-free pipette tips
- **One** of the following:
 - Homogenizer (page 67 and page 7) *or*
 - 1 mL RNase-free syringe with 18-21 gauge needle *or*
 - Rotor-stator homogenizer (page 8)
- 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)
-

Continued on next page

Purifying RNA from Yeast Cells, Continued

Preparing Lysis Buffer for Yeast Cells

Before beginning the lysis and homogenization steps, prepare a **fresh** amount of Lysis Buffer containing 1% 2-mercaptoethanol for each purification procedure. Add 10 μL 2-mercaptoethanol for each 1 mL Lysis Buffer.

Using the table below, determine the correct amount of Lysis Buffer needed for your specific lysis method.

Lysis method	Lysis Buffer (prepared with 2-mercaptoethanol)
Mechanical	0.5 mL
Enzymatic	200 μL

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 type of yeast cells and your sample size. Follow the manufacturer's instructions.
2. Transfer $\leq 5 \times 10^8$ log-phase yeast cells to an appropriately sized microcentrifuge tube and centrifuge at $500 \times g$ for 5 minutes at 4°C . Discard the supernatant.
3. Add up to 100 μL of Zymolase digestion buffer to the yeast cell pellet.
4. Incubate for 30–60 minutes at 30°C . The incubation time may vary depending on your yeast cell type and the enzyme used.
5. Add 200 μL Lysis Buffer prepared with 2-mercaptoethanol (see **Preparing Lysis Buffer for Yeast Cells** above) to the tube.
6. Vortex to mix thoroughly.
7. Centrifuge at $12,000 \times g$ for 2 minutes at room temperature to pellet cells.
8. Transfer the supernatant to a clean RNase-free microcentrifuge tube.

Proceed to **Binding, Washing, and Elution**, (page 40).

Continued on next page

Purifying RNA from Yeast Cells, Continued

Lysis and Homogenization: Mechanical Disruption

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

1. Transfer $\leq 5 \times 10^8$ log-phase yeast cells to an appropriately sized microcentrifuge tube and centrifuge at $500 \times g$ for 5 minutes at 4°C. Discard the supernatant.
2. Using a RNase-free mortar and pestle, crush approximately 10 grams of dry ice to a powder.
3. Add 0.5 mL of Lysis Buffer prepared with 2-mercaptoethanol (see **Preparing Lysis Buffer for Yeast Cells**, previous page) to the yeast cells and thoroughly resuspend.
4. Add the suspension from Step 3 drop-wise onto the crushed dry ice in the mortar. Grind the mixture with the RNase-free 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 (page 67) inserted in an RNase-free tube, and centrifuge at $12,000 \times g$ for 2 minutes at room temperature. Remove the Homogenizer when done, **or**
 - 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 $12,000 \times g$ for 2 minutes at room temperature, then transfer the supernatant to a clean RNase-free microcentrifuge tube, **or**
 - 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 the homogenate at $\sim 2,600 \times g$ for 5 minutes at room temperature, then transfer supernatant to clean RNase-free microcentrifuge tube.

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

Continued on next page

Purifying RNA from Yeast Cells, Continued

Binding, Washing, and Elution

Follow the steps below to bind, wash, and elute your yeast cell sample, prepared as described in your sample.

Note: There are different ethanol requirements depending on whether you performed enzymatic or mechanical disruption of the cells.

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

Mechanical Disruption: Add 1 volume 70% ethanol to each volume of yeast cell homogenate prepared by mechanical disruption.

2. Mix thoroughly by vortexing to disperse any visible precipitate that may form after adding ethanol.
3. Transfer up to 500 μL of your sample (including any remaining precipitate) to the Spin Cartridge (with a Collection Tube).
4. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. Discard the flow-through, and reinsert the Spin Cartridge in the same Collection Tube.
5. **Repeat** Steps 3–4 until the entire sample is processed.

Optional: If DNA-free total RNA is required, proceed to **On-column PureLink® DNase Treatment Protocol** (page 63).

6. Add 700 μL Wash Buffer I to the Spin Cartridge. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. Discard the flow-through **and** the Collection Tube. Insert the Spin Cartridge into a **new** Collection Tube.
7. Add 500 μL Wash Buffer II with ethanol (page 11) to the Spin Cartridge.
8. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. Discard the flow-through, and reinsert the Spin Cartridge into the same Collection Tube.
9. Repeat Steps 7–8 **once**.

Continued on next page

Purifying RNA from Yeast Cells, Continued

Binding, Washing, and Elution, continued

10. Centrifuge the Spin Cartridge at $12,000 \times g$ for 1 minute at room temperature to dry the membrane with attached RNA. **Discard** the Collection Tube and insert the Spin Cartridge into a Recovery Tube.
 11. Add $30 \mu\text{L}$ – $3 \times 100 \mu\text{L}$ RNase-Free Water to the center of the Spin Cartridge (see **Elution Parameters**, page 13).
 12. Incubate at room temperature for 1 minute.
 13. Centrifuge the Spin Cartridge for 2 minutes at $\geq 12,000 \times g$ at room temperature.
Note: If you are performing sequential elutions, collect all elutes into the same tube.
 14. Store your purified RNA (see page 4), or proceed to **Analyzing RNA Yield and Quality** (page 53) or to **DNase I Treatment After RNA Purification** (page 65).
-

Purifying RNA from Bacterial Cells

Introduction

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



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 RNA degradation prior to purification and for optimal yields of total RNA.
 - Ensure the pH of the lysozyme buffer is correct. Lower pH may result in reduced RNA yield.
-

Materials Needed

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

- 2-mercaptoethanol
 - 100% ethanol
 - 10% SDS (in RNase-free water), 0.5 μL /sample
 - Lysozyme solution, 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 $12,000 \times g$
 - 1.5 mL RNase-free microcentrifuge tubes
 - RNase-free pipette tips
 - Homogenizer (page 67 and page 7) *or* RNase-free syringe (1 mL) with 18-21 gauge needle *or* rotor-stator homogenizer (see page 8)
-

Preparing Lysozyme and Lysis Buffers for Bacterial Cells

Lysozyme Solution:

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

- 10 mM Tris-HCl (pH 8.0)
- 0.1 mM EDTA
- 1 mg lysozyme

Lysis Buffer:

Before beginning the lysis and homogenization steps, prepare a **fresh** amount of Lysis Buffer containing 1% 2-mercaptoethanol for each purification procedure. Add 10 μL 2-mercaptoethanol for each 1 mL Lysis Buffer.

Use 350 μL of freshly prepared Lysis Buffer for $\leq 1 \times 10^9$ bacterial cells.

Continued on next page

Purifying RNA from Bacterial Cells, Continued

Lysis and Homogenization

Use the following protocol to prepare your bacterial cells.

1. Harvest $\leq 1 \times 10^9$ log-phase bacterial cells and transfer them to an appropriately sized microcentrifuge tube. Centrifuge at $500 \times g$ for 5 minutes at 4°C to pellet cells. **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 10% SDS solution. Vortex to mix well.
4. Incubate cells in the tube for 5 minutes at room temperature.
5. Add 350 μL Lysis Buffer prepared with 2-mercaptoethanol (see previous page). Vortex to mix well.
6. Homogenize the cell lysate using **one** of the following options:
 - Transfer the lysate to a Homogenizer (page 67) inserted in an RNase-free tube, and centrifuge at $12,000 \times g$ for 2 minutes at room temperature. Remove the Homogenizer when done, **or**
 - 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 $12,000 \times g$ for 2 minutes at room temperature, then transfer the supernatant to a clean RNase-free microcentrifuge tube, **or**
 - 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 the homogenate at $\sim 2,600 \times g$ for 5 minutes at room temperature, then transfer the supernatant to clean RNase-free microcentrifuge tube.

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

Continued on next page

Purifying RNA from Bacterial Cells, Continued

Binding, Washing, and Elution

Follow the steps below to bind, wash, and elute the RNA from your bacterial cell sample.

1. Add 250 μL 100% ethanol to each volume of bacterial cell homogenate.
2. Mix thoroughly by vortexing to disperse any visible precipitate that may form after adding ethanol.
3. Transfer the sample (including any remaining precipitate) to a Spin Cartridge (with a Collection Tube).
4. Centrifuge both Spin Cartridge and Collection tube at $12,000 \times g$ for 15 seconds at room temperature. **Discard** the flow-through, and reinsert the Spin Cartridge in the same Collection Tube.

Optional: If DNA-free total RNA is required, proceed to **On-column PureLink[®] DNase Treatment Protocol** (page 63).

5. Add 700 μL Wash Buffer I to the Spin Cartridge. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. **Discard** the flow-through **and** the Collection Tube. Place the Spin Cartridge into a **new** Collection Tube.
6. Add 500 μL Wash Buffer II with ethanol (page 11) to the Spin Cartridge.
7. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. **Discard** the flow-through, and reinsert the Spin Cartridge into the same Collection Tube.
8. Repeat Steps 6–7 **once**.

Continued on next page

Purifying RNA from Bacterial Cells, Continued

Binding, Washing, and Elution, continued

9. Centrifuge the Spin Cartridge and Collection tube at $12,000 \times g$ for 1 minute at room temperature to dry the membrane with attached RNA. **Discard** the Collection Tube and insert the Spin Cartridge into a Recovery Tube.
 10. Add $30 \mu\text{L}$ – $3 \times 100 \mu\text{L}$ RNase-Free Water to the center of the Spin Cartridge (see **Elution Parameters**, page 13).
 11. Incubate at room temperature for 1 minute.
 12. Centrifuge the Spin Cartridge and Recovery Tube for 2 minutes at $\geq 12,000 \times g$ at room temperature.
Note: If you are performing sequential elutions, collect all elutes into the same tube.
 13. Store your purified RNA (see page 4), or proceed to **Analyzing RNA Yield and Quality** (page 53) or to **DNase I Treatment After RNA Purification** (page 65).
-

Purifying RNA from Liquid Samples/RNA Clean-Up

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 kit components:

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

Preparing Lysis Buffer for Liquid Samples

Before beginning the lysis and homogenization steps, prepare a fresh amount of Lysis Buffer containing 1% 2-mercaptoethanol for each purification procedure. Add 10 µL 2-mercaptoethanol for each 1 mL Lysis Buffer.

Use 1 volume of freshly prepared Lysis Buffer for each volume of liquid sample.

Continued on next page

Purifying RNA from Liquid Samples/RNA Clean-Up, Continued

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 Lysis Buffer prepared with 2-mercaptoethanol (see previous page) followed by the same volume of 96-100% ethanol (*e.g.*, to 1 mL of sample, add 1 mL of Lysis Buffer followed by 1 mL of ethanol).
2. Mix by vortexing or pipetting up and down 5 times (use RNase-free pipette tips).
3. Transfer up to 700 μ L of sample to a Spin Cartridge (with a Collection Tube).
4. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. **Discard** the flow-through and reinsert the Spin Cartridge into the same Collection Tube.
5. **Repeat** Steps 3–4 until the entire sample is processed.
Optional: If DNA-free total RNA is required, proceed to **On-column PureLink® DNase Treatment Protocol** (page 63).
6. **For cytoplasmic RNA extracts:** Add 700 μ L Wash Buffer I to the Spin Cartridge. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. **Discard** the flow-through **and** the Collection Tube. Insert the Spin Cartridge into a **new** Collection Tube.
For other samples: Proceed directly to Step 7.
7. Add 500 μ L Wash Buffer II with ethanol (page 11) to the Spin Cartridge. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. **Discard** flow-through and reinsert the Spin Cartridge into the same Collection Tube.
8. Repeat Step 7 **once**.

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Purifying RNA from Liquid Samples/RNA Clean-Up, Continued

Isolation of RNA from Liquid Samples, continued

9. Centrifuge the Spin Cartridge at $12,000 \times g$ for 1 minute at room temperature to dry the membrane with attached RNA. **Discard** the Collection Tube and insert the Spin Cartridge into a Recovery Tube.
 10. Add 30 μL – $3 \times 100 \mu\text{L}$ RNase-Free Water to the center of the Spin Cartridge (see **Elution Parameters**, page 13).
 11. Incubate at room temperature for 1 minute.
 12. Centrifuge the Spin Cartridge and Recovery Tube for 2 minutes at $\geq 12,000 \times g$ at room temperature.
Note: If you are performing sequential elutions, collect all elutes into the same tube.
 13. Store your purified RNA (see page 4), or proceed to **Analyzing RNA Yield and Quality** (page 53) or to **DNase I Treatment After RNA Purification** (page 65).
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Using TRIzol[®] Reagent with the PureLink[®] RNA Mini Kit

Introduction

This section provides instructions for using TRIzol[®] Reagent (page 67) in conjunction with the PureLink[®] RNA Mini Kit to isolate total RNA from samples that are difficult to lyse (e.g., fibrous animal tissues or plant tissue). This combined protocol also allows you to purify ultrapure total RNA for sensitive downstream applications such as qPCR or microarray analysis.

To obtain high-quality total RNA, be sure to follow the **Guidelines for Handling RNA** (page 4).



Important

The maximum binding capacity of the PureLink[®] RNA Mini Spin 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 such that each contains less than 1 mg of total RNA for each Spin Cartridge used.

Materials Needed

You will need the following items:

- TRIzol[®] Reagent (page 67)
 - Chloroform or 4-Bromoanisole
 - 2-mercaptoethanol
 - 96–100% ethanol or 70% ethanol (in RNase-free water), depending on protocol option used
 - Microcentrifuge capable of centrifuging 12,000 × g
 - 1.5 mL RNase-free microcentrifuge tubes
 - RNase-free pipette tips
-

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Using TRIzol[®] Reagent with the PureLink[®] RNA Mini Kit, Continued



TRIzol[®] Reagent contains phenol (toxic and corrosive) and guanidine isothiocyanate (an irritant), and may be a health hazard if not handled properly. Avoid direct contact with TRIzol[®] Reagent, as direct contact of skin, eyes, or respiratory tract with TRIzol[®] Reagent may cause chemical burns to the exposed area.

When working with TRIzol[®] Reagent, **always** work in a fume hood, and always wear a lab coat, gloves and safety glasses. Refer to the TRIzol[®] Reagent product insert for more details.

Contact your Environmental Health and Safety (EH&S) department for proper work and disposal guidelines.

Lysate Preparation with TRIzol[®] Reagent

Use TRIzol[®] Reagent to prepare lysates from various sample types as described below. Refer to the TRIzol[®] Reagent manual for more information.

Tissues

Homogenize 50–100 mg of tissue samples in 1 mL TRIzol[®] Reagent using a tissue homogenizer or rotor–stator.

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 an RNase-free pipette tip. The amount of TRIzol[®] Reagent required is based on the culture dish area (1 mL per 10 cm²) 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 × 10⁶ animal, plant, or yeast cells, or 1 × 10⁷ bacterial cells. Lyse cells by repetitive pipetting up and down.

Continued on next page

Using TRIzol[®] Reagent with the PureLink[®] RNA Mini Kit, Continued

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 with TRIzol[®] Reagent at room temperature for 5 minutes to allow complete dissociation of nucleoprotein complexes.
2. Add 0.2 mL chloroform or 50 μ L 4-Bromoanisole per 1 mL TRIzol[®] Reagent used. Shake the tube vigorously by hand for 15 seconds.

Note: Vortexing may increase DNA contamination of your RNA sample. Avoid vortexing if your downstream application is sensitive to the presence of DNA or perform an on-column DNase-digestion step during RNA purification (page 63) or after purification (page 65).

3. Incubate at room temperature for 2–3 minutes.
4. Centrifuge the sample at $12,000 \times g$ for 15 minutes at 4°C.

Note: After centrifugation, the mixture separates into a lower, red phenol-chloroform phase, an interphase, and a colorless upper aqueous phase which contains the RNA. The volume of the aqueous upper phase is ~600 μ L.

5. Transfer ~400 μ L of the colorless, upper phase containing the RNA to a fresh RNase-free tube.
6. Add an equal volume 70% ethanol to obtain a final ethanol concentration of 35%. Vortex to mix well.
7. Invert the tube to disperse any visible precipitate that may form after adding ethanol.

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

Continued on next page

Using TRIzol[®] Reagent with the PureLink[®] RNA Mini Kit, Continued

Binding, Washing, and Elution

1. Transfer ≤ 700 μL of sample (see previous page) to a Spin Cartridge (with a Collection Tube).
2. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. **Discard** the flow-through and reinsert the Spin Cartridge into the same Collection Tube.
3. **Repeat** Steps 1–2 until the entire sample is processed.
Optional: If DNA-free total RNA is required, proceed to **On-column PureLink[®] DNase Treatment Protocol** (page 63).
4. Add 700 μL Wash Buffer I to the Spin Cartridge. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. **Discard** the flow-through **and** the Collection Tube. Insert the Spin Cartridge into a **new** Collection Tube.
5. Add 500 μL Wash Buffer II with ethanol (page 11) to the Spin Cartridge.
6. Centrifuge at $12,000 \times g$ for 15 seconds at room temperature. **Discard** the flow-through, and reinsert the Spin Cartridge into the same Collection Tube.
7. Repeat Steps 5–6 **once**.
8. Centrifuge at $12,000 \times g$ for 1 minute at room temperature to dry the membrane. **Discard** the Collection Tube and insert the Spin Cartridge into a Recovery Tube.
9. Add 30–100 μL RNase-Free Water to the center of the Spin Cartridge (see **Elution Parameters**, page 13).
10. Incubate at room temperature for 1 minute.
11. Centrifuge the Spin Cartridge with the Recovery Tube for 2 minutes at $\geq 12,000 \times g$ at room temperature.
12. Store your purified RNA (see page 4), or proceed to **Analyzing RNA Yield and Quality** (page 53) or to **DNase I Treatment After RNA Purification** (page 65).

Continued on next page

TRIzol[®] Plus Total Transcriptome Isolation

Introduction

This section provides instructions for using TRIzol[®] Reagent (page 67) in conjunction with the PureLink[®] RNA Mini Kit to isolate total transcriptome RNA (total RNA, including small RNAs such as miRNA) from ≤ 10 mg of fresh or frozen tissue. This combined protocol also allows you to purify ultrapure total RNA for sensitive downstream applications such as qPCR or microarray analysis.

To obtain high-quality total RNA, be sure to follow the **Guidelines for Handling RNA** (page 4).

Materials Needed

You will need the following items:

- TRIzol[®] Reagent (page 67)
 - Chloroform
 - 100% ethanol
 - Microcentrifuge capable of centrifuging $\geq 12,000 \times g$
 - 1.5 mL RNase-free microcentrifuge tubes
 - RNase-free pipette tips
 - Rotor-stator homogenizer or Tissue homogenizer
-

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TRIzol[®] Plus Total Transcriptome Isolation

Continued



TRIzol[®] Reagent contains phenol (toxic and corrosive) and guanidine isothiocyanate (an irritant), and may be a health hazard if not handled properly. Avoid direct contact with TRIzol[®] Reagent, as direct contact of skin, eyes, or respiratory tract with TRIzol[®] Reagent may cause chemical burns to the exposed area.

When working with TRIzol[®] Reagent, **always** work in a fume hood, and always wear a lab coat, gloves and safety glasses. Refer to the TRIzol[®] Reagent product insert for more details.

Contact your Environmental Health and Safety (EH&S) department for proper work and disposal guidelines.

Lysate Preparation with TRIzol[®] Reagent

Before beginning, prepare all buffers and solutions according to protocol on page 11.

Use TRIzol[®] Reagent to prepare lysates from various sample types as described below. Refer to the TRIzol[®] Reagent manual for more information.

Tissues

Homogenize up to 10 mg of tissue in 1 mL TRIzol[®] Reagent using a rotor–stator homogenizer.

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 an RNase-free pipette tip. The amount of TRIzol[®] Reagent required is based on the culture dish area (1 mL per 10 cm²) and not on the number of cells present.

Suspension Cells

Harvest cells and pellet by centrifugation. Use 1 mL of TRIzol[®] Reagent per 1×10^6 animal cells. Lyse cells by repetitive pipetting up and down.

Continued on next page

TRIZol[®] Plus Total Transcriptome Isolation, Continued

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 with TRIZol[®] (previous page) at room temperature for 5 minutes to allow complete dissociation of nucleoprotein complexes.
2. Add 0.2 mL chloroform per 1 mL TRIZol[®] Reagent used. Cap and shake the tube vigorously by hand for 15 seconds.

Note: Vortexing may increase DNA contamination of your RNA sample. Avoid vortexing if your downstream application is sensitive to the presence of DNA.

3. Incubate at room temperature for 2–3 minutes.
4. Centrifuge the sample at $\geq 12,000 \times g$ for 15 minutes at 4°C.
Note: After centrifugation, the mixture separates into a lower, red phenol-chloroform phase, an interphase, and a colorless upper aqueous phase which contains the RNA. The volume of the aqueous upper phase is ~600 μ L.
5. Transfer the colorless, upper phase containing the RNA to a new RNase-free tube.
6. Add an equal volume 100% ethanol to obtain a final ethanol concentration of 50%. Vortex to mix well.
7. Invert the tube to disperse any visible precipitate that may form after adding ethanol.

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

Continued on next page

TRIzol[®] Plus Total Transcriptome Isolation, Continued

Binding, Washing, and Elution

Follow the steps below to bind, wash, and elute RNA from your sample.

Note: The total Transcriptome protocol does not contain a Wash Buffer I step.

1. Transfer ≤ 700 μL of sample (see previous page) to a Spin Cartridge (with a Collection Tube).
 2. Centrifuge at $\geq 12,000 \times g$ for 1 minute at room temperature. **Discard** the flow-through.
 3. Transfer any remaining sample to the Spin Cartridge and **repeat** Step 2, **once**.
 4. Discard the flow-through **and** the Collection Tube and insert the Spin Cartridge into a **new** Collection Tube.
 5. Add 500 μL Wash Buffer II with ethanol (page 11) to the center of the Spin Cartridge.
 6. Centrifuge at $\geq 12,000 \times g$ for 15 seconds at room temperature. **Discard** the flow-through.
 7. Repeat Steps 4–5 **once**.
 8. Centrifuge at $\geq 12,000 \times g$ for 1 minute at room temperature to dry the membrane with bound RNA. Discard the flow-through **and** the Collection Tube and insert the Spin Cartridge into a Recovery Tube.
 9. Add 30–100 μL RNase-Free Water to the center of the Spin Cartridge (see **Elution Parameters**, page 13).
 10. Incubate at room temperature for 1 minute.
 11. Centrifuge the Spin Cartridge with the Recovery Tube for 2 minutes at $\geq 12,000 \times g$ at room temperature.
Note: The dead volume of the PureLink[™] Mini Kit Spin Cartridge is ~ 2 μL . An elution volume of 12 μL will result in a final elute volume of 10 μL (see **Elution Parameters**, page 13).
 12. Store your purified RNA (see page 4), or proceed to **Analyzing RNA Yield and Quality** (page 53).
-

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 (page 67) 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 OD₂₆₀ 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) = OD₂₆₀ × [40 μg / (1 OD₂₆₀ × 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 OD₂₆₀ of 0.188 was obtained. The amount of RNA in the sample is determined as shown below:

Total RNA (μg) =

$$0.188 \times [40 \mu\text{g} / (1 \text{ OD}_{260} \times 1 \text{ mL})] \times 12.5 \times 0.15 = 14.1 \mu\text{g}$$

Continued on next page

Analyzing RNA Yield and Quality, Continued

RNA Quality

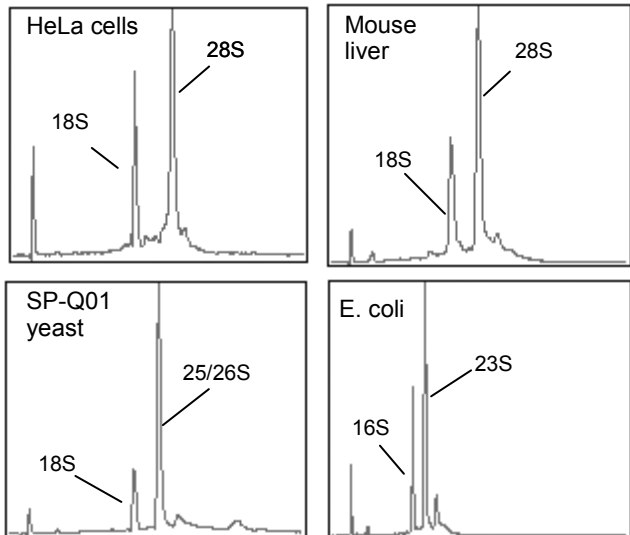
Typically, total RNA isolated using the PureLink™ RNA Mini Kit has an $OD_{260/280}$ of >1.8 when samples are diluted in Tris-HCl (pH 7.5). An $OD_{260/280}$ of >1.8 indicates that RNA is reasonably clean of proteins and other UV chromophores that could either interfere with downstream applications or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

Agarose gel electrophoresis of RNA isolated using the PureLink™ RNA Mini 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.

Bioanalyzer Analysis of RNA Quality

The quality of purified total RNA can also 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™ RNA Mini Kit.

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™ RNA Mini Kit. RNA quantitation was performed using UV absorbance at 260 nm.

Sample type	Sample	Amount	Average Yield (µg)
Animal Cells	HeLa cells	1 × 10 ⁶	15–20
	293 cells	1 × 10 ⁶	20–25
Animal Tissue	Rat liver	10 mg	60
		100 mg	300
	Rat brain	10 mg	6
		100 mg	90
	Rat spleen	10 mg	58
		100 mg	320
Calf thymus	10 mg	48	
	100 mg	350	
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	<i>S. cerevisiae</i>	1 × 10 ⁷	1.8
		5 × 10 ⁸	125

Troubleshooting

Introduction

Refer to the table below to troubleshoot any problems you may encounter with the PureLink™ RNA Mini Kit.

Problem	Cause	Solution
Clogged Homogenizer	Highly viscous lysate (<i>e.g.</i> , 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 RNA yield	Incomplete lysis and homogenization	Ensure that 10 µL of 2–mercaptoethanol was added per 1 mL of Lysis Buffer. Perform all steps at room temperature unless directed otherwise. Decrease the amount of starting material used, or increase volume of Lysis Buffer. 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 Lysis Buffer 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 –80°C or in liquid nitrogen immediately after harvesting.

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
Low RNA yield, continued	Ethanol not added to Wash Buffer II	Be sure that ethanol was added to Wash Buffer II as directed on page 11.
	Incorrect elution conditions	Add RNase-free water and perform incubation for 1 minute before centrifugation. Follow the recommendations under Elution Parameters (page 13). To recover more RNA, perform a second elution step.
RNA degraded	RNA contaminated with RNase	Use RNase-free pipette tips with aerosol barriers. Change gloves frequently. Swipe automatic pipettes with RNase AWAY™ solution after washing the 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 -80°C or in liquid nitrogen. Frozen samples must remain frozen until Lysis Buffer was added. Perform the lysis quickly after adding Lysis Buffer.
	Tissue very rich in RNases (e.g., rat pancreas)	RNA isolated from tissue rich in RNases may require the addition of RNase inhibitors/inactivators to protect the RNA from degradation, or use a larger volume of Lysis Buffer. Elute samples in 100% formamide. If the RNA is used for mRNA isolation of Northern blots, elute in 0.1% SDS.

Continued on next page

Troubleshooting, Continued

Problem	Cause	Solution
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 on page 63) or after purification (see protocol page 65).
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 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 $A_{260}/_{280}$ ratio	Sample was diluted in water; non-buffered water has variable pH (Wilfinger <i>et al.</i> , 1997)	Use 10 mM Tris-HCl (pH 7.5) to dilute sample for OD measurements.

Appendix

On-column PureLink® DNase Treatment Protocol

Introduction

This section provides a protocol for removing DNA from your sample using PureLink® DNase (page 67), while the RNA is bound on the Spin Cartridge. This protocol includes the PureLink® DNase treatment, followed by steps to complete the washing and elution of your RNA (refer to your sample-specific protocol for the appropriate step at which to perform this on-column DNase treatment).

Note: Alternatively, you may perform a DNase I (page 67) digestion of the RNA sample *after* purification (page 65). However, this may result in reduced RNA yield.

PureLink® DNase

PureLink® DNase is optimized for use with the PureLink® RNA Mini Kit. It is designed to be used specifically for on-column digestion of DNA during critical RNA purification procedures for downstream application procedures that require DNA-free total RNA.

Resuspending PureLink® DNase

Resuspend the PureLink® DNase by dissolving the lyophilized DNase in 550 µL RNase-Free Water (supplied with PureLink® DNase).

Store at 4°C for short-term storage. For long-term storage, prepare aliquots of the DNase and store at -20°C. Avoid repeat freezing and thawing. Thawed DNase stocks may be stored at 4°C for up to six weeks.

Preparing PureLink® DNase

Before beginning, prepare PureLink® DNase for on-column treatment, add the following components (supplied with PureLink® DNase) to a clean, RNase-free microcentrifuge tube. Prepare 80 µL per sample.

<u>Component</u>	<u>Volume</u>
10X DNase I Reaction Buffer	8 µL
Resuspended DNase (~3U/µL)	10 µL
<u>RNase Free Water</u>	<u>62 µL</u>
Final Volume	80 µL

Continued on next page

On-column PureLink[®] DNase Treatment Protocol, Continued

On-column PureLink[®] DNase Treatment and Purification

Use the After binding your RNA to the membrane of the Spin Cartridge, perform this on-column PureLink[®] DNase treatment to purify DNA-free total RNA (refer to your sample-specific protocol for the appropriate step). Continue this protocol to complete the washing and elution steps of your RNA.

Note: There is a wash step after binding and prior to the addition of PureLink[™] DNase.

1. Add 350 μ L Wash Buffer I to the Spin Cartridge containing the bound RNA (see sample-specific protocol). Centrifuge at 12,000 \times g for 15 seconds at room temperature. **Discard** the flow-through **and** the Collection Tube. Insert the Spin Cartridge into a **new** Collection Tube.
2. Add 80 μ L PureLink[®] DNase mixture (prepared as described on previous page) directly onto the surface of the Spin Cartridge membrane.
3. Incubate at room temperature for 15 minutes.
4. Add 350 μ L Wash Buffer I to the Spin Cartridge. Centrifuge at 12,000 \times g for 15 seconds at room temperature. **Discard** flow-through **and** the Collection Tube and insert the Spin Cartridge into a **new** Collection Tube.
5. Add 500 μ L Wash Buffer II with ethanol (page 11) to the Spin Cartridge.
6. Centrifuge at 12,000 \times g for 15 seconds at room temperature. **Discard** flow-through and reinsert the Spin Cartridge into the same Collection Tube.
7. Repeat Steps 5–6, **once**.

Continued on next page

On-column PureLink[®] DNase Treatment Protocol, Continued

On-column PureLink[®] DNase Treatment, continued

8. Centrifuge the Spin Cartridge at $12,000 \times g$ for 1 minute to dry the membrane with bound RNA. **Discard** Collection Tube and insert the Spin Cartridge into a Recovery Tube.
 9. Add 30 μL –100 μL RNase–Free Water to the center of the Spin Cartridge.
 10. Incubate at room temperature for 1 minute.
 11. Centrifuge Spin Cartridge and Recovery Tube for 1 minute at $\geq 12,000 \times g$ at room temperature.
 12. Store your purified RNA (see page 4), or proceed to **Analyzing RNA Yield and Quality** (page 53).
-

DNase I Treatment After RNA Purification

Introduction

This section provides a protocol for removing DNA from your sample after RNA purification. You may also perform an on-column DNase I digestion during the purification procedure (page 63).

DNase I, Amplification Grade

The following protocol uses DNase I, Amplification Grade. See page 67 for ordering information.

DNase-treatment of purified RNA

Following RNA purification using the PureLink® RNA Mini Kit, perform the following DNase I digestion procedure:

1. Add the following items to your tube containing the purified RNA:

<u>Component</u>	<u>Volume</u>
RNA (up to 10 µg/µL)	up to 8 µL
10X DNase I Buffer	1 µL
RNase-Free Water	add to 8 µL
DNase I, Amplification Grade (1 unit/µL)	1 µL
Final Volume	10 µL

2. Incubate for 15 minutes at room temperature.
3. Use either heat inactivation or purification to remove the DNase I.

For heat inactivation:

Add 1 µL of 25 mM EDTA solution to the reaction and heat at 65°C 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.

For removal DNase I by Purification:

Proceed to **Purifying RNA from Liquid Samples**, page 46.

Additional Products

Additional Products

The following products are also available from Life Technologies.

For details, visit www.lifetechnologies.com or contact **Technical Support** (page 67).

RT-PCR and qRT-PCR Products

Product	Quantity	Catalog No.
PureLink™ 96 RNA Lysis Buffer	750 mL	12173-022
PureLink™ DNase	50 preps	12185-010
DNase I, Amplification Grade (1 unit/μL)	100 units	18068-015
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 Platinum® Two-Step qRT-PCR Kit	100 reactions	11734-050

Other Products

Product	Quantity	Catalog No.
Homogenizer	50 pack	12183-026
RNase AWAY®	250 mL	10328-011
TRIzol® Reagent	100 mL 200 mL	15596-026 11596-018
TRIzol® LS Reagent	100 mL 200 mL	10296-010 10296-028
TRIzol® Max™ Bacterial RNA Isolation Kit	100 kit	16096-020
Plant RNA Reagent	100 mL	12322-012
0.1–2 Kb RNA Ladder	75 μg	15623-100
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

Technical Support

Obtaining Support

For the latest services and support information for all locations, go to www.lifetechnologies.com

At the website, you can:

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

Safety Data Sheets (SDSs) are available at www.lifetechnologies.com/support

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