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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life technologies™

Contents

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

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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	≤5 × 10^7 cells	13
Animal tissue	≤200 mg	21
Plant tissue	≤250 mg	28
Whole blood	≤0.2 mL	34
Yeast cells	≤5 × 10^8 cells	37
Bacterial cells	≤1 × 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 µL
Wash Tube Capacity:	2.0 mL
Centrifuge Compatibility:	Capable of >12,000 × *g*
Elution Volume: **Elution**	30 µL–3 × 100 µL (see **Parameters,** 13)
RNA Yield:	Varies with sample type and quality (see page 59)

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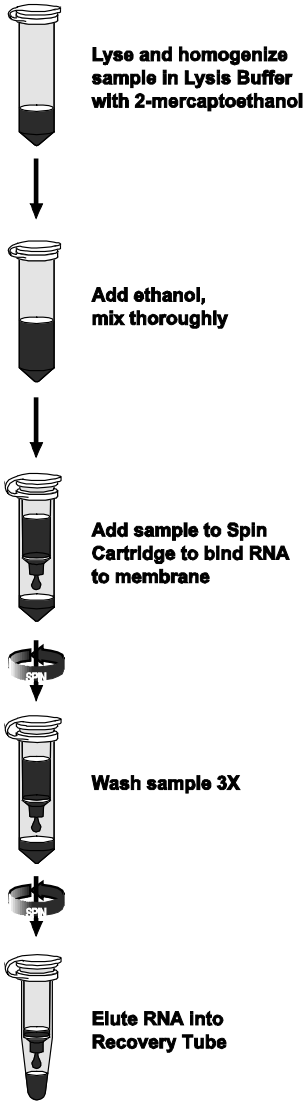
Workflow

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

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 4ºC 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.

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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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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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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	• Homogenizer • Syringe and needle • Rotor-stator	Rotor-stator is required for homogenization of $>10^7$ cells.
Animal Tissue: Frozen or Fresh Fibrous	Pestle with microcentrifuge tube (≤10 mg tissue)	• Homogenizer • Syringe and needle	--
	Mortar and pestle in liquid nitrogen (10-100 mg tissue)	• Homogenizer • Syringe and needle	--
	Rotor-stator (≤200 mg tissue)		Rotor-stator lyses and homogenizes simultaneously and can be used with all tissue amounts up to 200 mg.
Animal Tissue: Fresh Soft	Pestle with microcentrifuge tube (≤100 mg tissue)	• Homogenizer • Syringe and needle	--
	Rotor-stator (≤200 mg tissue)		Rotor-stator lyses and homogenizes simultaneously and can be used with all tissue amounts up to 200 mg.

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Sample Lysis and Homogenization, continued

Sample Type	Lysis Options	Homogenization Options	Comments
Plant Tissue: Frozen or Fresh Fibrous	Mortar and pestle in liquid nitrogen	• Homogenizer • Rotor-stator	We recommend using a mortar and pestle with liquid nitrogen for more complete lysis than 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	• Homogenizer • Syringe and needle • Rotor-stator	Recommended for kinetic experiments.
Bacteria	Digestion with lysozyme, vortexing	• Homogenizer • Syringe and needle • Rotor-stator	--
Liquid samples	Lysis Buffer, vortexing		--

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 Heath 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 cm^2) and not on the number of cells present.

Suspension Cells

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

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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 µL 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 × *g* for 15 minutes at 4ºC.

   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 µL.
5. Transfer ~400 µL 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.

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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 × *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 × *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 × *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 × *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 ≥12,000 × *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

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

Calculate the amount of total RNA using the following formula:

Total RNA (µg) = 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 OD_{260} of 0.188 was obtained. The amount of RNA in the sample is determined as shown below:

Total RNA (µg) =

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

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RNA Quality

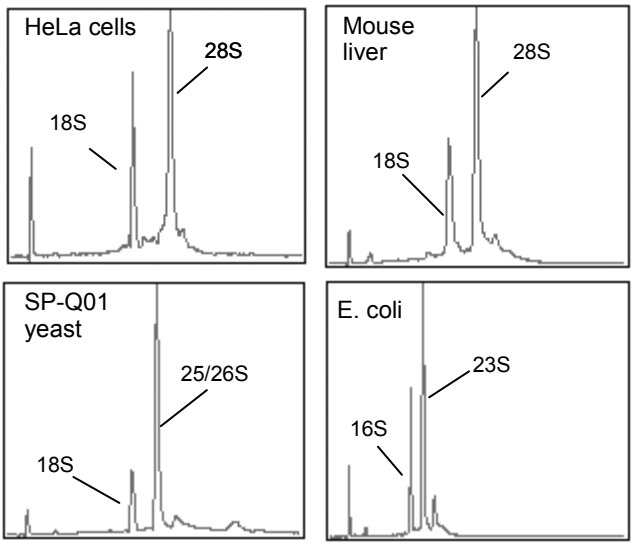
Typically, total RNA isolated using the PureLink™ RNA Mini Kit has an $OD_{260/280}$ of >1.8 when samples are diluted in Tris-HCl (pH 7.5). An $OD_{260/280}$ of >1.8 indicates that RNA is reasonably clean of proteins and other UV chromophores that could either interfere with downstream applications or 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	*S. cerevisiae*	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 (*e.g.*, calf thymus)	Homogenize sample with rotor–stator homogenizer.
Clogged RNA Spin Cartridge	Incomplete homogenization or dispersal of precipitate after ethanol addition	Follow protocol guidelines for each sample type and amount. Clear homogenate and remove any particulate or viscous material by centrifugation and use only the supernatant for subsequent loading on to the RNA Spin Cartridge. Completely disperse any precipitate that forms after adding ethanol to the homogenate.
Low 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 –80ºC or in liquid nitrogen immediately after harvesting.

Continued on next page

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 –80ºC 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 *et al.*, 1997)	Use 10 mM Tris-HCl (pH 7.5) to dilute sample for OD measurements.

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

Safety Data Sheets (SDS)

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

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.lifetechnologies.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

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