TōTALLY RNA™ Kit

(Part Number AM1910)

Protocol

I. Introduction 1

A. Background

B. Materials Provided with the Kit and Storage

C. Required Materials Not Provided With the Kit

D. Related Products Available from Applied Biosystems

II. Sample Disruption and Homogenization 4

A. Equipment and Solution Preparation

B. Animal or Plant Tissue Samples

C. Cultured Eukaryotic Cells

D. Bacteria

E. Yeast

III. RNA Isolation 10

A. Preparation of Lysate for RNA Isolation

B. Phenol:Chloroform:IAA Extraction

C. Acid Phenol Extraction

D. Isopropanol Precipitation of the RNA

E. Long Term Storage of RNA

IV. Assessing RNA Yield and Quality 14

A. Assessing RNA Yield and Purity

B. Denaturing Agarose Gel Electrophoresis

V. Troubleshooting 18

A. Low RNA Yield

B. Problems During Denaturing Gel Electrophoresis

C. RNA Degradation

D. Contaminants in RNA; RNA Inhibits Enzymatic Reactions

VI. Optional RNA Clean-up Procedures 23

A. (optional) LiCl Precipitation

B. Removal of Trace DNA Contamination with DNase I

VII. Appendix 25

A. Recipes

B. References

C. Safety Information

D. Quality Control

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Literature Citation: When describing a procedure for publication using this product, please refer to it as the TōTALLY RNA™ Kit.

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I. Introduction

A. Background

NOTE

The isopropanol is not provided due to environmental concerns associated with the packaging that would be required for co-shipment of alcohol and phenol solutions.

Classical RNA isolation procedures are based on disruption of cells in guanidinium thiocyanate/cationic detergent solutions, followed by organic extraction and alcohol precipitation of the RNA. Guanidinium solutions are powerful reagents for the inactivation of endogenous RNases, but the tendency for DNA, heme, and other pigments and impurities to copurify with the RNA can be a problem. The Ambion TōTALLY RNA™ Total RNA Isolation Kit is designed to remove undesirable impurities from cellular RNA without sacrificing speed, convenience, or RNA yield. Samples are lysed in a guanidinium based lysis solution and are then extracted sequentially with Phenol:Chloroform:IAA and Acid-Phenol:Chloroform. The RNA is then precipitated with isopropanol, and reagents are provided for an optional lithium chloride (LiCl) precipitation as well. The kit includes all the reagents (except isopropanol) required for isolation of total RNA from up to 10 g of tissue or about 10^9 cultured cells, and also includes ancillary reagents for analysis and storage of the RNA.

B. Materials Provided with the Kit and Storage

CAUTION

Phenol is a highly corrosive chemical capable of causing severe burns. Wear protective clothing, gloves, and safety glasses at all times when handling the Phenol:Chloroform:IAA and the Acid-Phenol:Chloroform. We recommend using a chemical safety hood for phenol extractions. See the Material Data Safety Sheets available at www.ambion.com/techlib/msds for additional precautions.

Amount	Component	Storage
1 mL	Formaldehyde Load Dye	–20°C
100 mL	Phenol:Chloroform:IAA	4°C
100 mL	Acid-Phenol:Chloroform	4°C
15 mL	Sodium Acetate Solution 3.0 M sodium acetate pH 4.5	4°C
50 mL	Lithium Chloride Precipitation Solution 7.5 M lithium chloride/50 mM EDTA	4°C
5 mL	5 M Potassium Acetate	4°C
100 mL	Denaturation Solution	room temp
50 mL	Elution Solution (0.1 mM EDTA)	any temp*

* Store Elution Solution at –20°C, 4°C, or room temp.

C. Required Materials Not Provided With the Kit

Apparatus for tissue grinding and homogenization

Liquid nitrogen, mortar and pestle, dry ice

- For grinding tissue: not all samples will need to be ground in liquid nitrogen, see Table 1 on page 6. For more information see (www.Ambion.com—click Technical Resources, then choose The Basics/RNA Isolation, or Technical Bulletins 183, 177, or Tips from the Bench/several relevant topics)

Tissue homogenizer

- Electronic rotor-stator tissue homogenizers are recommended if available.
- Manual tissue homogenizers or grinders: conical ground glass tissue grinders work well, dounce-type smooth glass homogenizers may also be acceptable.

Reagents

- Isopropanol: ACS grade or better
- (Optional) Ethanol: ACS grade or better 95–100% ethanol
- (Optional) Liquid nitrogen (for disruption of frozen and/or hard tissue)

Centrifugation equipment

- Centrifuge capable of 10,000 x g for large-scale preps, or a micro-centrifuge capable of 12,000 x g for small-scale preps.
- Centrifuge tubes compatible with phenol/chloroform (glass, polypropylene, or polyallomer), capable of withstanding centrifugal forces of 12,000 x g, with securely fitting closures.

Materials for analysis of RNA

- Spectrophotometer or Agilent Bioanalyzer (http://www.chem.agilent.com/Scripts/PDS.asp?lPage=51)
- Reagents and apparatus for preparation and electrophoresis of denaturing agarose gels (see section IV.B on page 15 for instructions).

D. Related Products Available from Applied Biosystems

RNA*later*® Solution P/N AM7020, AM7021	RNA*later* Tissue Collection: RNA Stabilization Solution is an aqueous sample collection solution that stabilizes and protects cellular RNA in intact, unfrozen tissue and cell samples. RNA*later* Solution eliminates the need to immediately process samples or to freeze samples in liquid nitrogen. Samples can be submerged in RNA*later* Solution for storage at RT, 4°C, or –20°C without jeopardizing the quality or quantity of RNA that can be obtained.
RNase*Zap*® Solution P/N AM9780, AM9782, AM9784	RNase*Zap* RNase Decontamination Solution is simply sprayed, poured, or wiped onto surfaces to instantly inactivate RNases. Rinsing twice with distilled water will eliminate all traces of RNase and RNase*Zap* Solution.
Millennium™ Markers and BrightStar® Biotinylated Millennium™ Markers P/N AM7150 and AM7170	Ambion's Millennium™ Markers are designed to provide very accurate size determination of single-stranded RNA transcripts from 0.5 to 9 kb and can be used in any Northern protocol. They are a mixture of 10 easy-to-remember sizes of in vitro transcripts: 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6 and 9 kb.
DNA-*free*™ Reagents P/N AM1906	DNase treatment and removal reagents. This product contains Ambion's ultra-high quality RNase-free DNase I and reaction buffer for degrading DNA. It is ideal for removing contaminating DNA from RNA preparations. A novel reagent for removing the DNase without the hassles or hazards of phenol extraction or alcohol precipitation is also included.
Electrophoresis Reagents see our web or print catalog	Ambion offers gel loading solutions, agaroses, acrylamide solutions, powdered gel buffer mixes, nuclease-free water, and RNA and DNA molecular weight markers for electrophoresis. Please see our catalog or our website (www.ambion.com) for a complete listing as this product line is always growing.

II. Sample Disruption and Homogenization

The first and most important step in any RNA isolation procedure is the rapid disruption and homogenization of the sample. Sample disruption must be rapid enough to expose the intracellular contents to the Denaturation Solution before endogenous RNases have a chance to degrade the RNA.

Once homogenized, lysates can be processed immediately or stored frozen at –80°C for several months.

A. Equipment and Solution Preparation

Make sure that the Denaturation Solution is completely dissolved

It is common for a precipitate to form in the Denaturation Solution. Before using it, check the bottle carefully to see if a precipitate has formed during storage, and if so, heat the solution to 37°C, swirling the bottle occasionally, until the precipitate goes back into solution.

Lab bench and pipettors

Before working with RNA, clean the lab bench, and pipettors with an RNase decontamination solution such as Ambion RNase*Zap*® RNase Decontamination Solution.

Gloves and RNase-free technique

Wear laboratory gloves at all times during this procedure and change them frequently. They will protect you from the reagents, and they will protect the RNA from nucleases that are present on skin.

Use RNase-free pipette tips to handle the kit reagents.

Washing/sterilization of equipment

The equipment used for tissue disruption/homogenization should be washed well with detergent and rinsed thoroughly. Baking is unnecessary, because the Denaturation Solution will inactivate any low level RNase contamination.

IMPORTANT

If samples will be ground in a mortar and pestle, pre-chill the equipment in dry ice or liquid nitrogen.

Be sure to use RNase-free labware for all manipulations downstream of the Acid-Phenol:Chloroform extraction, because once the RNA is precipitated out of the Denaturation Solution, it will be highly susceptible to degradation by RNase.

B. Animal or Plant Tissue Samples

For a good yield of intact RNA, it is very important to harvest tissue quickly and to limit the time between obtaining tissue samples and inactivating RNases in step 3 below.

1. Harvest tissue

Harvest tissue and remove as much extraneous material as possible, for example remove adipose tissue from heart, and remove gall bladder from liver. The tissue can be perfused with cold PBS if desired to eliminate some of the red blood cells.

2. Cut larger tissue into small pieces

If necessary, quickly cut the tissue into pieces small enough for either storage or disruption. Weigh the tissue sample (this can be done later for samples that will be stored in RNA*later*® Solution).

3. Inactive RNases by one of the following methods:

- Drop the sample into 5–10 volumes of RNA*later* Solution—tissue must be cut to ≤0.5 cm in at least one dimension. (See the RNA*later* Solution Protocol for detailed instructions.)
- Disrupt the sample in Denaturation Solution (see instructions in step 5 on page 6). This option is only appropriate for fresh tissue samples that are soft to medium consistency.
- Freeze the sample in liquid nitrogen—tissue pieces must be small enough to freeze in a few seconds. When the liquid nitrogen stops churning it indicates that the tissue is completely frozen. Once frozen, remove the tissue from the liquid nitrogen and store it in an airtight container at –80°C.
  Very hard or fibrous tissues (e.g., bone and heart), and tissues with a high RNase content must typically be frozen in liquid nitrogen and ground to a powder for maximum RNA yield.

4. Prepare tissue disruption equipment/supplies

The method used to disrupt tissue samples depends on the nature of the tissue, the storage method, and the size of the sample; Table 1 (below) shows guidelines for tissue disruption methods.

Table 1.Tissue Disruption Methods

Tissue consistency	Sample storage method	Suggested disruption method
All frozen samples	Frozen	Freeze and grind in liquid N_2
Very hard	Any storage method	Freeze and grind in liquid N_2 or use a more rigorous method like disruption in a bead mill or a freezer mill
Hard or RNase-rich	Freshly dissected or stored in RNA*later* Solution	Freeze and grind in liquid N_2
Soft to medium	Freshly dissected or stored in RNA*later* Solution	Electric or manual homogenizer
Soft, small pieces (<0.5 cm^3)	Frozen	Electric or manual homogenizer

NOTE

Ambion's website has comprehensive information on tissue disruption. Go to www.ambion.com—click on the document search button and type "disruption" into the search engine.

5. Thoroughly homogenize sample in the greater of 200 µL or 10 µL/mg of tissue Denaturation Solution

Thoroughly homogenize sample in the greater of 200 µL or 10 µL/mg of tissue Denaturation Solution following the instructions below for samples stored in RNA*later*®, fresh, or frozen samples.

5a. Preparing samples stored in Ambion RNA*later*® or RNA*later*-ICE Solutions

Samples in RNA*later* Solution can usually be homogenized by following the instructions for fresh tissue in step 5b (next). Extremely tough/fibrous tissues in RNA*later* Solution may need to be frozen and pulverized according to the instructions for frozen tissue (step 5c) in order to achieve good cell disruption.

If the samples were immersed in RNA*later* Solution and then frozen at –80°C, simply thaw samples at room temperature before starting. Blot excess RNA*later* Solution from samples, and weigh them before following the instructions for fresh tissue below.

5b. Fresh animal or plant tissue sample preparation (soft to medium consistency tissues)

a. If the sample weight is unknown, weigh the sample.

b. Aliquot the greater of 200 µL or 10 µL/mg of tissue Denaturation Solution into the homogenization vessel.
For example, if your sample weighs 500 mg, use 5 mL Denaturation Solution. For very small samples use at least 200 µL of Denaturation Solution; this will be >10 volumes.

c. Mince large samples (≥2 cm^2) rapidly in cold PBS, then remove the PBS before proceeding to the next step (PBS recipe in section VII.A.3 on page 25).

d. Drop samples into the Denaturation Solution, and process to homogeneity. If available, use a motorized rotor-stator homogenizer (e.g., Polytron).
Some tissues may need to be frozen in liquid nitrogen and powdered in a mortar and pestle before homogenization to obtain maximum RNA yield and quality.

5c. Frozen, hard-consistency, or RNase-rich tissue sample preparation

After removing the tissue from the freezer, it is important to process it immediately without allowing any thawing. This is necessary because as cells thaw, ice crystals rupture cellular compartments, releasing RNase. By processing samples before they thaw, RNases can be inactivated by the Denaturation Solution before they are released from their cellular compartments.

a. If the sample weight is unknown, weigh the sample.

b. Aliquot the greater of 200 µL or 10 µL/mg of tissue Denaturation Solution into a a wide-mouth container. (After grinding the tissue in liquid nitrogen, the frozen powder will be transferred to this container—we find that plastic weigh boats work well.)
For example, if your sample weighs 500 mg, use 5 mL Denaturation Solution. For very small samples use at least 200 µL of Denaturation Solution; this will be >10 volumes.


NOTE

Using an electronic rotor-stator homogenizer, small pieces of relatively soft frozen tissues (i.e. <0.5 cm^3) can often be added directly to the Denaturation Solution without first grinding it in a mortar and pestle.

c. Grind frozen tissue to a powder with liquid nitrogen in a pre-chilled mortar and pestle.
Some researchers grind frozen tissue in a coffee grinder with dry ice. Also, sample fragments larger than ~100 mg can be shattered with a hammer.

d. Using a pre-chilled metal spatula, scrape the powdered tissue into the premeasured Denaturation Solution, then mix rapidly.

e. Transfer the slush to a vessel for homogenization and process the mixture to homogeneity. If available, use a motorized rotor-stator homogenizer.

Once homogenized, lysates can be processed immediately or stored frozen at –80°C for several months.

C. Cultured Eukaryotic Cells

1. Collect the cells and remove the culture medium

Suspension cells: pellet the cells at low speed, and discard the culture medium.

Adherent cells: Do one of the following

- Aspirate and discard the culture medium from the culture vessel.
- Trypsinize cells to detach them from the growing surface (following the method employed in your lab for the cell type).

2. Add 10 mL Denaturation Solution to 5 x 10^7 to 5 x 10^8 cells and lyse the cells

a. Add 10 mL Denaturation Solution to 5 x 10^7 to 5 x 10^8 cells and vortex or pipette the lysate up and down several times to completely lyse the cells and to obtain a homogenous lysate. Cells will lyse immediately upon exposure to the solution.

b. For adherent cells, collect the lysate with a rubber spatula.

3. Frozen cell pellets

IMPORTANT

Instead of using frozen cell pellets, lyse fresh cells as described above if possible, and freeze the lysate.

Grind frozen cell pellets in liquid nitrogen in a mortar and pestle as described for frozen tissue (step 5b on page 9). This is necessary because as cells thaw, ice crystals rupture both interior and exterior cellular compartments, releasing RNase.

Once homogenized, lysates can be processed immediately or stored frozen at –80°C for several months.

D. Bacteria

1. Harvest 10^8–10^9 cells by centrifugation. (For many strains, this corresponds to ~3 mL of an overnight culture.) Remove as much of the culture medium as possible.
2. (optional) Enzyme pre-treatment:
   - Resuspend cells in 100 µL TE (10 mM Tris-HCl, 1 mM EDTA) with 1 mg/mL lysozyme or lysostaphin.
   - Incubate 5 min at room temperature for lysozyme, or 15 min at 37°C for lysostaphin to degrade cell envelopes.
3. Add 300 µL Denaturation Solution for up to 3 mL of culture starting volume. (The exact volume of Denaturation Solution is usually not critical and can be adjusted according to user experience.)
4. Vortex vigorously to thoroughly lyse cells.

E. Yeast

1. Start with a logarithmically growing culture (A_{600}~1–2); pellet cells and rinse once with nuclease-free water.
   Cells that are grown past the logarithmic phase will usually be more difficult to lyse.
2. Add 300 µL Denaturation Solution per up to 3 mL of culture starting volume.

3. Remove a 2 µL aliquot as a baseline for an absorbance reading at 260 nm. Yeast are difficult to lyse, and an easy way to monitor lysis is by looking for an increase in the A_{260} resulting from the release of nucleic acids.

NOTE

The glass beads will not interfere with RNA isolation, they will remain with the lower, organic phase in the next step of the procedure.

4. Add ~150–200 µL of 0.4–0.5 mm glass beads, and vortex vigorously for 1 min intervals, checking the A_{260} of 2 µL samples after each interval. (Dilute the samples of lysate in 1 mL water to read their absorbance). Lysis will normally be complete after 2–4 rounds of vortexing (1 min each). The A_{260} reading should increase sharply as lysis begins and then level off indicating that lysis is complete.

III. RNA Isolation

A. Preparation of Lysate for RNA Isolation

1. Reduce the viscosity of the lysate if necessary

Lysate should be somewhat viscous, but if the solution is very viscous, or contains gelatinous material (which is probably genomic DNA), then it should be sonicated, homogenized in a rotor-stator homogenizer, or passed through a 25 gauge syringe needle several times until the viscosity is reduced. It may be necessary to reduce viscosity by adding more Denaturation Solution and homogenizing with an electronic tissue disrupter.

To continue with the procedure the lysate should be about as viscous as a typical enzyme solution in 50% glycerol.

2. (optional) Centrifuge 2–3 min at top speed in a microcentrifuge to remove debris

This centrifugation removes any debris that may be present in the lysate. Most preparations will not have any insoluble material after thorough homogenization.

B. Phenol:Chloroform:IAA Extraction

1. Measure the *Starting Volume* of the lysate

Measure the volume of the lysate. This volume will be referred to as the ***Starting Volume***.

Transfer lysate to a vessel resistant to organic solvents with a capacity that is slightly more than twice the volume of the lysate.

2. Extract with 1 Starting Volume Phenol: Chloroform: IAA

a. Add 1 Starting Volume of Phenol:Chloroform:IAA to the lysate. Be sure to use the organic phase of the Phenol:Chloroform:IAA which lies under the thin upper layer of aqueous buffer.

CAUTION

Phenol is highly corrosive. Wear protective clothing, gloves and safety glasses when handling phenol.

b. Shake or vortex vigorously for 1 minute.

c. Store lysate + phenol on ice for the following times depending on the amount of solution, then centrifuge at 10,000–12,000 x g for the indicated time preferably at 4°C:

Tube Size	Incubation on ice	Centrifugation at 10,000–12,000 x g
1.5–2 mL	5 min	5 min
10–15 mL	10 min	15 min
larger containers	15 min	15 min

3. Transfer the aqueous phase to a new vessel

a. Transfer the upper, aqueous phase to new vessel of the same size being careful to avoid the material at the aqueous/organic interface.

b. Measure the volume of the aqueous phase. Do not be concerned if the volume of the lysate is slightly increased compared to the Starting Volume.

c. (optional) To thoroughly recover the remainder of the aqueous phase from the organic phase and the interface, withdraw the remaining aqueous material from the top of the interphase into a pipet tip; it is okay if some of the interface material enters the pipet tip also. Let the phases separate in the pipet tip for several seconds, and allow as much of the viscous interface material as possible to drip back into the tube with the phenol before transferring the aqueous portion to a fresh tube. Centrifuge the recovered aqueous material for ~1 min to separate the phases. Then, using a clean pipet tip transfer the aqueous portion to the container with the rest of the prep.

C. Acid Phenol Extraction

1. Add 1/10 volume Sodium Acetate Solution and mix well

Add 1/10 Aqueous Phase volume of Sodium Acetate Solution to the phenol extracted lysate (from step A.3 above). Mix by shaking or inversion for about 10 sec.

2. Extract with 1 Starting Volume Acid-Phenol: Chloroform

a. Add 1 Starting Volume of Acid-Phenol:Chloroform. Be sure to use the organic phase of the Acid-Phenol:Chloroform which lies under the aqueous buffer floating on top.
Do not add more than 1 Starting Volume even if the volume of the lysate is greater than the starting volume.

b. Shake or vortex vigorously for 1 min.

c. Store lysate + phenol on ice and centrifuge as in step 2.c on page 10.

3. Transfer the aqueous phase to a new vessel

IMPORTANT

Vessels used at this step must be RNase-free because the protective effect of the Denaturation Solution will be removed after the RNA is precipitated out in the next steps.

a. Transfer the upper, aqueous phase to a new RNase-free vessel with a capacity of at least twice the volume of the lysate at this point in the procedure. Be careful to avoid material from the interface of the aqueous and organic phases.

b. Measure the volume of the aqueous phase. Do not be concerned if the volume of the lysate is slightly different than the Starting Volume.

c. (optional) Recover the remainder of the aqueous phase from the organic phase and the interface as described in step A.3.c on page 11.

D. Isopropanol Precipitation of the RNA

1. Add an equal volume of isopropanol, mix well

Add an equal volume (final volume from step C.3) of isopropanol to the RNA prep and mix well.


NOTE

*A fine white precipitate that forms **immediately** upon addition of the isopropanol may indicate the presence of undesirable material which can be removed before storing the prep at –20°C. (See section V.6.7.c on page 22 for further details.)*

2. Place at –20°C for ≥30 min to 1 hr

Place the preparation at –20°C for at least 30 min for 1.5–2 mL tubes, or at least 1 hr for larger tubes.

A white flocculent precipitate may form, indicating precipitation of the RNA. At any time after a visible precipitate forms, or after overnight storage at –20°C (even if no precipitate is visible), proceed to step 3.


NOTE

This is a stopping point in the procedure, and the preparation may be stored at this stage for a period of up to several days if desired.

3. Recover the RNA by centrifugation

Centrifuge the precipitation mixture at 10,000–12,000 x g for 15 min in microfuge tubes, or for 20 min if the mixture is in larger tubes.

4. Carefully remove and discard the supernatant

Carefully remove the supernatant solution. Pellets may not adhere tightly to the walls of some tubes, so be careful if you decant to remove the supernatant. For 1.5–2 mL tubes, we suggest removing the supernatant by gentle aspiration with a very fine pipette tip or a drawn-out Pasteur pipette and bulb.

Re-spin the tube briefly and remove any residual fluid by aspiration with a fine-tipped pipette.

5. (optional) Ethanol wash

Residual salts can be removed by washing the pellet in 70% ethanol as follows:

a. Add room temperature 70% ethanol to the RNA pellet; use ~300 µL for microfuge tubes or 2–3 mL for RNA pellets in larger tubes.

b. Gently vortex or flick the tube with a finger for ~0.5–3 min.

c. Recover the RNA by centrifugation for 5–10 min at low speed (~3,000 x g: 7,500 rpm in a microcentrifuge, or 5,500 rpm in an SS34 rotor) at room temp or 4°C.

d. Carefully and thoroughly remove ethanol supernatant; pellets may not adhere tightly to the tubes. Re-spin tubes briefly and remove residual ethanol by aspiration with a fine-tipped pipette.

6. Resuspend RNA in DEPC Water/ EDTA

Resuspend pellets in the desired volume of DEPC Water/ EDTA. Typically 100 µL–1 mL per 100 mg of starting material (or ~10^7 cells) will yield a reasonable RNA concentration.

If necessary, heat the solution to 55–70°C, and vortex occasionally to dissolve the RNA. Typically RNA from small scale RNA preparations will go into solution after a few minutes, but RNA pellets from large scale preps may require more time to dissolve.

E. Long Term Storage of RNA

It is unwise to subject RNA to repeated cycles of freeze-thawing (RNA Methodologies, a Laboratory Guide, 1992). To avoid repeated freeze-thawing, total RNA samples should be stored in small aliquots at –70°C or –80°C. A small amount of EDTA (0.1 mM) should be present during storage to chelate divalent cations, which can catalyze RNA breakage. If degradation problems are encountered upon prolonged storage, it may be desirable to store the RNA as an ethanol precipitate (i.e., add 2 volumes of ethanol to the prep in aqueous solution). The RNA can be recovered by centrifugation, after adjusting the salt concentration to 0.25 M with potassium acetate (use the 5 M Potassium Acetate provided with the kit). Alternatively, RNA can be precipitated with LiCl (see section VI.A on page 25), or with potassium acetate and ethanol and resuspended in formamide for long term storage at –20°C (Chomczynski, 1992).

IV. Assessing RNA Yield and Quality

A. Assessing RNA Yield and Purity

RNA yield

Spectrophotometry

The concentration of an RNA solution can be determined by measuring its absorbance at 260 nm (A_{260}) using a spectrophotometer. With a traditional spectrophotometer, dilute an aliquot of the RNA 1:50–1:100 in TE (10 mM Tris-HCl pH 8, 1 mM EDTA), and read the absorbance. (Be sure to zero the spectrophotometer with the TE used for sample dilution.) The buffer used for dilution need not be RNase-free, since slight degradation of the RNA will not significantly affect its absorbance.

NanoDrop spectrophotometers are more convenient—no dilutions or cuvettes are needed, just measure 1.5 µL of the RNA sample directly.

To determine the RNA concentration in µg/mL, multiply the A_{260} by the dilution factor and the extinction coefficient (1 A_{260} = 40 µg RNA/mL).

A_{260} x dilution factor x 40 = µg RNA/mL

Be aware that any contaminating DNA in the RNA prep will lead to an overestimation of yield, since all nucleic acids absorb at 260 nm.

Fluorometry

If a fluorometer or a fluorescence microplate reader is available, Molecular Probes' RiboGreen® fluorescence-based assay for RNA quantitation is a convenient and sensitive way to measure RNA concentration. Follow the manufacturer's instructions for using RiboGreen.

RNA quality

Microfluidic analysis

Microfluidic systems such as the Agilent® 2100 bioanalyzer with Caliper's RNA LabChip® Kits provide better quantitative data than conventional gel analysis for characterizing RNA. When used with Ambion RNA 6000 Ladder (P/N AM7152), this system can provide a fast and accurate size distribution profile of RNA samples. Follow the manufacturer's instructions for performing the assay.

The 28S to 18S rRNA ratio is often used as an indicator of RNA integrity. Total RNA isolated from fresh and frozen mammalian tissues using this kit usually has a 28S to 18S rRNA ratio of >1.2.

Using a bioanalyzer, the RIN (RNA Integrity Number) can be calculated to further evaluate RNA integrity. A metric developed by Agilent, the RIN analyzes information from both rRNA bands, as well as information contained outside the rRNA peaks (potential degradation products) to provide a fuller picture of RNA degradation states. Search for "RIN" at Agilent's website for information:

www.chem.agilent.com

Spectrophotometry

An effective measure of RNA purity is the ratio of absorbance readings at 260 and 280 nm; it should fall in the range of 1.8 to 2.1. Even if an RNA prep has a ratio outside of this range, it may function well in common applications such as RT-PCR, Northern blotting, and RNase protection assays.

B. Denaturing Agarose Gel Electrophoresis

The overall quality of an RNA preparation may be assessed by electrophoresis on a denaturing agarose gel; this will also give some information about RNA yield. A denaturing gel system is suggested because most RNA forms extensive secondary structure via intramolecular base pairing, and this prevents it from migrating strictly according to its size. Be sure to include a positive control RNA on the gel so that unusual results can be attributed to a problem with the gel or a problem with the RNA under analysis. RNA molecular weight markers, an RNA sample known to be intact, or both, can be used for this purpose.

Ambion NorthernMax® reagents for Northern Blotting include everything needed for denaturing agarose gel electrophoresis. These products are optimized for ease of use, safety, and low background, and they include detailed instructions for use.

An alternative to using the NorthernMax reagents is to use the procedure described below. This denaturing agarose gel method for RNA electrophoresis is modified from "Current Protocols in Molecular Biology", Section 4.9 (Ausubel et al., eds.). It is more time-consuming than the NorthernMax method, but it gives similar results.

1. Prepare the gel

a. Heat 1 g agarose in 72 mL water until dissolved, then cool to 60°C.

b. Add 10 mL 10X MOPS running buffer, and 18 mL 37% formaldehyde (12.3 M).

CAUTION

Formaldehyde is toxic through skin contact and inhalation of vapors. Manipulations that involve formaldehyde should be done in a chemical fume hood.

Table 2. 10X MOPS Running Buffer

Concentration	Component
0.4 M	MOPS, pH 7.0
0.1 M	sodium acetate
0.01 M	EDTA

c. Pour the gel using a comb that will form wells large enough to accommodate at least 25 µL.

d. Assemble the gel in the tank, and add enough 1X MOPS running buffer to cover the gel by a few millimeters. Then remove the comb.

2. Prepare the RNA sample

a. To 1–3 µg RNA, add 0.5–3 volumes Formaldehyde Load Dye (included in kit).

- To simply check the RNA on a denaturing gel, as little as 0.5 volumes Formaldehyde Load Dye can be used, but to completely denaturate the RNA, e.g. for Northern blots, use 3 volumes of Formaldehyde Load Dye.
- Ethidium bromide can be added to the Formaldehyde Load Dye at a final concentration of 10 µg/mL.
  Some size markers may require significantly more than 10 µg/mL ethidium bromide for visualization. To accurately size your RNA, however, it is important to use the same amount of ethidium bromide in all the samples (including the size marker) because ethidium bromide concentration affects RNA migration in agarose gels.

b. Heat denature samples at 65–70°C for 5–15 min.
Denaturation for 5 min is typically sufficient for simply assessing RNA on a gel, but a 15 min denaturation is recommended when running RNA for a Northern blot. The longer incubation may be necessary to completely denature the RNA.

3. Electrophoresis

Load the gel and electrophorese at 5–6 V/cm until the bromophenol blue (the faster-migrating dye) has migrated at least 2–3 cm into the gel, or as far as 2/3 the length of the gel.

4. Results

Visualize the gel on a UV transilluminator. (If ethidium bromide was not added to the Formaldehyde Load Dye, the gel will have to be post-stained and destained.)

Figure 1 shows a typical denaturing agarose gel containing RNA isolated with the ToTALLY RNA Kit. The 28S and 18S ribosomal RNA bands should be fairly sharp, intense bands (size is dependent on the organism from which the RNA was obtained). The intensity of the upper band

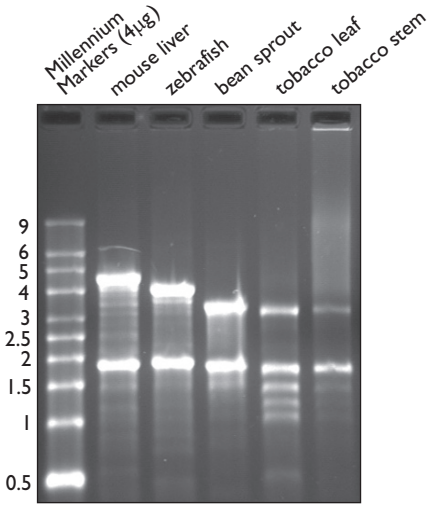

Figure 1. Total RNA isolated using the TōTALLY RNA™ Kit

Total RNA was isolated from the indicated sources, and approximately 2 µg was run on a 1% denaturing agarose gel stained with ethidium bromide. The smaller rRNA bands in the lanes with leaf samples are derived from organelles such as chloroplasts and other plastids.

should be about twice that of the lower band. Smaller, more diffuse bands representing low molecular weight RNAs (tRNA and 5S ribosomal RNA) may be also present. It is normal to see a diffuse smear of ethidium bromide staining material migrating between the 18S and 28S ribosomal bands, probably comprised of mRNA and other heterogeneous RNA species. DNA contamination of the RNA preparation (if present) will be evident as a high molecular weight smear or band migrating above the 28S ribosomal RNA band, or sometimes as ethidium bromide staining material that does not migrate out of the well. Degradation of the RNA will be reflected by smearing of ribosomal RNA bands.

V. Troubleshooting

The most common problems in RNA isolation are low yields, recovery of degraded RNA, and copurification of undesirable contaminants. Each of these problems is discussed below.

A. Low RNA Yield

Sample disruption was suboptimal

Inadequate sample disruption is a frequent source of problems in RNA isolation, especially for tough tissues such as muscle and kidney. Tissue disruption can be improved by snap freezing tissue in liquid nitrogen, then grinding it to a powder in a mortar and pestle under liquid nitrogen. The powder should then be quickly mixed with Denaturation Solution and homogenized with a rotor/stator homogenizer.

For some tissues even grinding in liquid nitrogen is not sufficient to disrupt the tissue; these extremely tough tissues will require harsher disruption techniques for example in a bead mill or in a freezer mill. For more information, visit www.Ambion.com—click Technical Resources, then choose The Basics/RNA Isolation, or Technical Bulletins 183, 177, or Tips from the Bench/several relevant topics. Technical Bulletins can also be requested through our Technical Services Department (contact info is on the back cover of this booklet).

RNA is not abundant in the tissue from which it was isolated

Expected yields of RNA vary widely between tissues. If you are accustomed to working with tissues such as liver or kidney where RNA is plentiful, you may have unrealistically high expectations of RNA yields from tissues such as muscle or brain.

The RNA pellet was not completely solubilized

Centrifuge the preparation briefly and look for particles of undissolved material, which may appear as small amber gelatinous flecks. Solubilization may be improved by further incubation of the preparation at 55–70°C, with intermittent vortexing. Do not allow the RNA pellet to dry out completely after removing the supernatant fluid subsequent to precipitation, or solubilization will become difficult, if not impossible.

The sample was too dilute in the Denaturation Solution

Poor RNA yield may be improved in some cases by keeping the preparation more concentrated. If a certain sample type routinely gives low RNA yield, try using only 5 volumes of Denaturation Solution per unit mass of tissue, and scale down the subsequent reagents accordingly. Significant errors in weighing small tissue samples (less than about 50 mg) are introduced if the samples are extremely wet, so it is a good idea to blot tissues dry just before weighing them.

Loss of RNA at the interface during organic extractions

Poor RNA yield can also be caused by excessive loss of material at the aqueous/phenol interface during the 2 organic extraction steps. A rapid "interface respin" procedure may be used to maximize recovery of the aqueous phase. This is described in step III.B.3.c on page 11.

B. Problems During Denaturing Gel Electrophoresis

Problems with gel electrophoresis can cause RNA to appear to be degraded. Consider these suggestions if you suspect that electrophoresis was not optimal.

IMPORTANT

Gel problems can be ruled out by running an aliquot of "positive control" RNA, i.e. an archived, intact RNA sample, on the same gel as the RNA preparation being evaluated for the first time.

Ribosomal RNA (rRNA) is overloaded

Running more than about 5 µg of RNA in a single lane may cause smiling and/or smearing of the rRNA bands. rRNA comprises about 80% of total RNA, so if 5 µg of total RNA is loaded in a gel lane, there will be about 1 µg and 3 µg of RNA in the 18S and 28S rRNA bands respectively.

Samples are incompletely denatured

To *completely* denature RNA, the sample should be diluted with at least 3 volumes of Formaldehyde Load Dye and then incubated in a 65°C water bath for at least 15 min. A 65°C cabinet type incubator works well, but somewhat longer incubation times may be required due to the lower heat transfer capacity of air. After incubation, transfer the samples immediately to an ice bath. Samples are stable on ice for at least 20 min, or long enough to add ethidium bromide to the samples if desired and load them on the gel. If an interruption occurs, the samples may be incubated at 65°C again without ill effects.

Gel was run too fast

Smearing may occur if gels are run at more than 5–6 volts/cm as measured between the electrodes. For example, if the distance between the electrode wires in the electrophoresis chamber measures 15 cm, the gel should be run at a constant 75 volts.

Electrophoresis buffer was depleted

For long runs (>3 hr) the buffer may be circulated to avoid the formation of pH gradients in the gel. This can be accomplished in various ways: manual circulation of the buffer every 15–30 min throughout the run (be sure samples have migrated into the gel first), continuous circulation of the buffer from one chamber to the other with a pump, or continuous circulation of the buffer using magnetic stir bars placed in both chambers.

Gel or gel apparatus was contaminated with RNase

RNase contamination of the gel running equipment, reagents, or supplies can cause RNA degradation while the gel is running. To decontaminate equipment, we recommend using Ambion RNA*Zap*® RNase Decontamination Solution following the instructions provided.

C. RNA Degradation

Improper handling of tissue

It is extremely important to inactive RNases as quickly as possible after sample collection to avoid RNA degradation. When samples are obtained from sacrificed animals or cadavers, it is also important to limit the time between death and sample collection for the best yield of high quality RNA.

Frozen tissue thawed before immersion in Denaturation Solution

It is essential that frozen tissue stays frozen until it is disrupted in Denaturation Solution.

If the tissue is frozen in small pieces (i.e. <0.5 cm^3), and it will be processed with an electronic rotor-stator homogenizer (Polytron type), it can often be dropped directly in a vessel containing Denaturation Solution and processed before it has a chance to thaw or to freeze the Denaturation Solution. This shortcut generally only works for relatively soft tissues.

When powdering tissue in a mortar and pestle, it is important to pre-chill the mortar and pestle, and to keep adding small amounts of liquid nitrogen during grinding so that the tissue never thaws, even partially. Once the tissue is completely powdered, it should be mixed with the Denaturation Solution quickly before any of the powder can thaw. It may be convenient to scrape the frozen powder into a plastic weigh boat containing the volumes of Denaturation Solution.

Samples that are very high in RNase may require procedure modification for isolation of intact RNA

- Extra organic extractions can be included using the Phenol:Chloroform:IAA and Acid-Phenol:Chloroform provided with the kit, followed by a final extraction with an equal volume of chloroform:isoamyl alcohol(24:1 or 49:1).
- Degradation may be minimized by ultracentrifugation of the RNA through a cesium chloride cushion, as an alternative to alcohol precipitation (Groppe and Morse, 1993).
- Degradation can sometimes be minimized by reducing the quantity of starting material (tissue or cells) used for the isolation (Kamdar and Evens, 1992). In such cases it may help to also use a lower mass of tissue relative to volume of Denaturation Solution, for example use 0.5–0.75 gm tissue per 10 mL Denaturation Solution.
- For problematic tissues, greater care may be needed to avoid contamination with interface material during the organic extraction steps (in other words if may be advisable to sacrifice yield to avoid degradation).

Include a radiolabeled "tracer RNA" to troubleshoot degradation problems

For troubleshooting RNA degradation problems, it may be useful to add a radiolabeled synthetic RNA transcript to the Denaturation Solution along with the sample. If intact ^{32}P labeled RNA is recovered at the final step (as assessed by TCA precipitation or autoradiography), then the Denaturation Solution was adequate to inactivate RNases released from the disrupted cells, and the degradation probably occurred before starting the ToTALLY RNA procedure.

Material that precipitates immediately after adding isopropanol can cause RNA degradation

Occasionally a degradation problem that seems to be associated with a contaminant in lung tissue is observed, which results in formation of a precipitate immediately upon addition of the isopropanol at step D.1 on page 12. The material differs from RNA in the following ways:

- It forms ***immediately*** after the isopropanol is added.
- It is a finer, less flocculent precipitant than RNA.
- Upon vortexing and briefly spinning the tube, the material forms a sheet on the side of the tube, instead of a pellet at the bottom (like RNA).

If this precipitate forms in a prep ***immediately*** after the isopropanol is added in step D.1 on page 12, remove it by spinning the tube briefly (~10 seconds). Transfer the prep (in isopropanol) to a fresh tube and continue with the procedure (at step D.2 on page 12). Although some RNA may be lost along with the contaminant, a lower yield of intact RNA is preferable to a good yield of degraded RNA.

D. Contaminants in RNA; RNA Inhibits Enzymatic Reactions

DNA contamination

Digest the prep with DNase I (RNase-free)

Instructions for digesting RNA with DNase I are shown in section *VI.B. Removal of Trace DNA Contamination with DNase I* on page 25. Alternatively, DNA-*free*™ can be used for this application.

Precipitate the RNA with LiCl

IMPORTANT

LiCl precipitation is not efficient when the RNA concentration is below 0.2 µg/µL.

LiCl precipitation (section VI.A on page 25) will selectively precipitate RNAs longer than 200 nt, and will leave behind most of the DNA, carbohydrates, and protein.

High molecular weight band on a gel that is not DNA

Some RNA preps show faint bands of ethidium bromide staining material migrating just above the 28S ribosomal RNA band, which is ***not*** comprised of DNA, as it is not removed by DNase treatment. The origin of this material is unclear, but it does not seem to adversely affect the quality of the RNA.

Other contaminants

Any of the following suggestions will further purify RNA after the ToTALLY RNA procedure.

a. Precipitate the RNA with LiCl

LiCl precipitation (see section VI.A on page 25) will selectively precipitate RNAs ≥200 nt, and will leave behind DNA, carbohydrates, and protein.

b. Salts

Wash RNA pellets with 75% ethanol (step D.5 on page 12) to remove residual salts which could interfere with enzymatic reactions.

c. Protein contamination

Protein contamination is suspected if the RNA exhibits an $A_{260/280}$ ratio below ~1.7. To remove protein, extract the RNA with an equal volume of Phenol:Chloroform:IAA (see step B.2 on page 10). The resultant RNA may be extracted with an equal volume of chloroform:isoamyl alcohol (24:1 or 49:1) to remove any residual phenol. Despite these efforts, the A_{260}:A_{280} ratio may sometimes remain below 1.7, especially for RNA isolated from tissues such as liver and kidney. For most applications, a low A_{260}:A_{280} ratio will probably not affect the results. We have used total RNA with A_{260}:A_{280} ratios ranging from 1.4 to 1.8 with good results in RNase protection assays, Northern blots, in vitro translation experiments, and RT-PCR.

d. Intractable contaminants

For removal of intractable contaminants, it may be desirable to recover the RNA from the Denaturation Solution by centrifugation through a cesium chloride cushion (Chirgwin et al. 1979, Molecular Cloning p. 7.19–7.22, Groppe and Morse 1993).

VI. Optional RNA Clean-up Procedures

A. (optional) LiCl Precipitation

IMPORTANT

The concentration of RNA should be at least 0.2 µg/µL to assure efficient precipitation. Also, LiCl precipitation will not quantitatively precipitate small RNAs such as tRNA and 5S ribosomal RNA.

Lithium chloride precipitation removes carbohydrates and gross DNA contamination.

1. Mix the RNA well with one-half volume LiCl Precipitation Solution.
2. Incubate at –20°C for at least 30 min.
3. Microcentrifuge 15 min at top speed.
4. Carefully remove and discard the supernatant.
5. Wash pellet with cold 70% ethanol, re-centrifuge, aspirate away the supernatant.
6. Air dry the pellet.

B. Removal of Trace DNA Contamination with DNase I

Trace DNA contamination can be enzymatically removed using DNase I. Note that under optimal conditions, PCR can detect a single DNA molecule, so even DNase treatment cannot always guarantee removal of genomic DNA below the level detectable by PCR.

Ambion TURBO DNA-*free*™ Kit

The Ambion TURBO DNA-*free*™ Kit (P/N AM1907) includes Ambion TURBO DNase™ (patent pending), the first DNase I enzyme engineered for superior DNA digestion. TURBO DNA-*free* also simplifies the process of inactivating the DNase without the risk incurred by heating the RNA, or the inconvenience of extracting with phenol/chloroform. TURBO DNA-*free* is the method of choice for eliminating contaminating DNA prior to RT-PCR; to use TURBO DNA-*free*, follow the instructions provided with the product.

Using your own DNase treatment reagents

DNase digestion buffer

DNase treatment can be carried out in the buffer supplied or in a buffer recommended by the manufacturer of the enzyme; most restriction enzyme buffers can also be used. DNase I works well in a large range of salt and pH conditions. The enzyme requires magnesium (~5 mM) for optimal activity.

Amount of DNase

- To remove small amounts of DNA, DNase I should be used at approximately 10 Units/mL RNA.
- To treat severe DNA contamination, use 1 Unit DNase I per µg of contaminating DNA.

Incubation conditions
Incubate DNase digestions at 37°C for 30 min.

Inactivate DNase I by one of the following methods:

- Add EDTA to 5 mM, heat to 75°C for 10 min
- Add EDTA to 20 mM
- Extract with phenol/chloroform, and alcohol precipitate the RNA.

VII. Appendix

A. Recipes

1. 10X TBE

TBE is generally used at 1X final concentration for preparing gels and/or for gel running buffer.

IMPORTANT

Do not treat TBE with diethylpyrocarbonate (DEPC).

Concentration	Component	for 1 L
0.9 M	Tris base	109 g
0.9 M	Boric Acid	55 g
20 mM	0.5 M EDTA solution	40 mL

Dissolve with stirring in about 850 mL nuclease-free water. Adjust the final volume to 1 L.

Alternatively, Ambion offers nuclease-free solutions of 10X TBE (P/N AM9863, AM9865) and ready-to-resuspend powdered 10X TBE packets (P/N AM9864). Both are made from of ultrapure molecular biology grade reagents.

2. RNase-free water

a. Add DEPC to 0.05% to double-distilled, deionized water (i.e. add 0.5 mL per liter of water).

b. Stir well, incubate several hours to overnight at 37°C or 42°C.

c. Autoclave 2 L or smaller volumes for at least 45 min. After autoclaving, the scent of DEPC should be either undetectable or only very slightly detectable.

3. Phosphate Buffered Saline (PBS)

Concentration	Component	for 1 L
137 mM	NaCl	8 g
2.7 mM	KCl	0.2 g
10 mM	Na_2HPO_4	1.42 g
1.8 mM	KH_2PO_4	0.25 g

Dissolve the components in about 800 ml dH_2O. Adjust the pH to 7.4 with HCl. Adjust the volume to 1 L. Sterilize by autoclaving. Store at room temperature.

B. References

Chirgwin, JM, Przybyla, AE, MacDonald, RJ, and Rutter, WJ (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **24**: 5294–5299.

Chomczynski, P and Sacchi, N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162**: 156-159.

Chomczynski, P, (1992) Solubilization in Formamide Protects RNA From Degradation. *Nucleic Acids Res* **20**: 3791.

Groppe, J, and Morse, D (1993) Isolation of full-length RNA templates for reverse transcription from tissues rich in RNase and proteoglycans. *Anal Biochem* **210**:337–343.

Kamdar, S and Evans, R (1992) Modifications of the Guanidine Hydrochloride Procedure for the Extraction of RNA: Isolation From a Variety of Tissues and Adherent/Non-adherent Cell Types. *BioTechniques* **12**: 632–637.

Nolan C, Editor (1989) *Molecular Cloning, A Laboratory Manual,* 2nd ed., Cold Springs Harbor Laboratory Press.

Puissant, C and Houdebine, LM (1990) An improvement of the single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *BioTechniques* **8**: 148–149.

Robert E Farrell Jr, Editor (1993) *RNA Methodologies, A Laboratory Guide for Isolation and Characterization* Academic Press, Inc.

Monstein, HJ, Nylander, G, and Chen, D (1995) RNA extraction from gastrointestinal tract and pancreas by a modified Chomczynski and Sacchi method. *BioTechniques* **19**:339–344

C. Safety Information

The MSDS for any chemical supplied by Applied Biosystems or Ambion is available to you free 24 hours a day.

IMPORTANT

For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.

To obtain Material Safety Data Sheets

- Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: www.ambion.com/techlib/msds
- Alternatively, e-mail your request to: MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery.
- For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

D. Quality Control

Functional Testing

All of the individual components of the kit have been subject to rigorous quality control analysis. All components are also tested in a functional RNA isolation procedure.

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

RNase activity
Meets or exceeds specification when a sample is incubated with labeled RNA and analyzed by PAGE.

Nonspecific endonuclease activity
Meets or exceeds specification when a sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity
Meets or exceeds specification when a sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.