PROTOCOL

ambion® | RNA
by life technologies™

RiboPure™ Kit

life technologies™

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RiboPure™ Kit

(Part Number AM1924)

Protocol

I. Introduction

IMPORTANT

Before using this product, read and understand the "Safety Information" in the appendix in this document.

A. Product Description and Procedure Overview

The Ambion RiboPure™ Kit is designed for rapid purification of high quality RNA from tissue samples or cultured cells. The RiboPure Kit combines the robust lysis/denaturant, TRI Reagent®, with glass-fiber filter purification methodology.

TRI Reagent lysis and glass-fiber filter clean-up

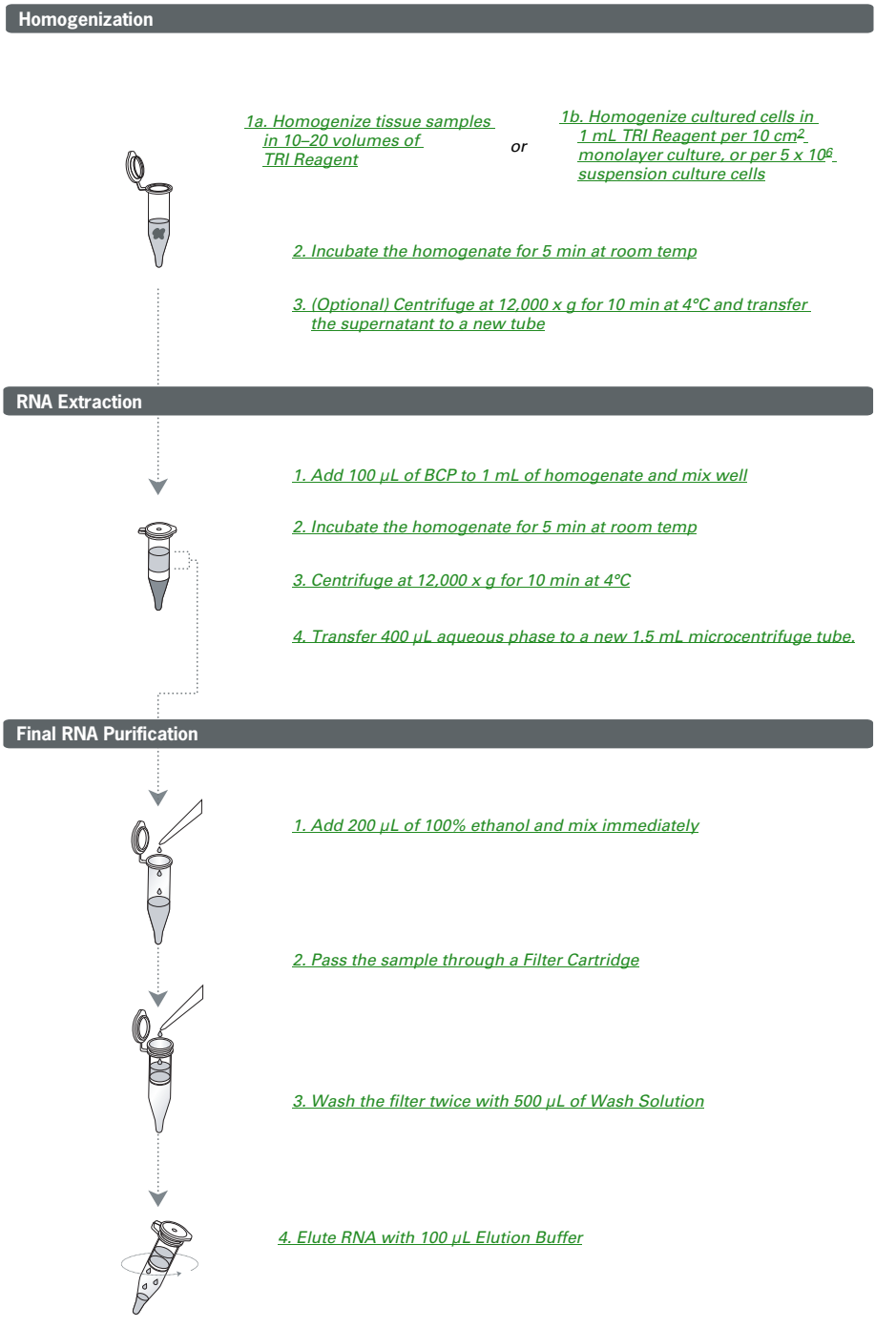
The RiboPure procedure is fast (<30 min) and straightforward (Figure 1 on page 2). Samples are homogenized in TRI Reagent, a monophasic solution containing phenol and guanidine thiocyanate, which rapidly lyses cells and inactivates nucleases. Addition of bromochloropropane (BCP) results in separation of the homogenate into aqueous and organic phases. RNA partitions to the aqueous phase, while DNA and protein remain in the interphase and organic phase. The RNA is isolated from the aqueous phase by binding to a glass-fiber filter. After two on-filter washes to remove contaminants, the RNA is eluted from the filter with a low salt buffer.

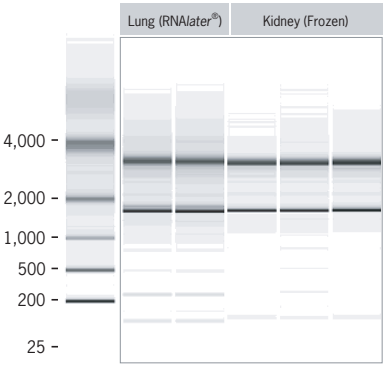
High yield and high-quality RNA

The RiboPure procedure is suitable for 5–100 mg fresh or frozen tissue, 0.1–20 x 10^6 cultured cells, or 10 cm^2 monolayer culture area. The procedure is compatible with tissues that have been stored in Ambion RNA*later*® Solution. Total RNA yield is typically 100–500 µg per 100 mg of tissue, depending on the type of tissue. Figure 2 on page 3 illustrates the performance of the RiboPure Kit with frozen mouse kidney tissue, and lung tissue that had been stored in RNA*later* Solution. In this experiment, the 28S:18S rRNA ratio of the recovered RNA, one indicator of RNA integrity, was consistently ≥1.2.

The recovered RNA is suitable for use in all common downstream applications, including cDNA synthesis, real-time and end-point RT-PCR, microarray analysis, Northern blots, and RNase protection assays.

Figure 1. RiboPure™ Procedure Overview





Yield (mg)	123.24	121	288.28	210.8	233.12
A_{260}/A_{280}	2.07	1.97	2.05	2.15	2.05
28S/18S rRNA	1.2	1.2	1.7	1.6	1.7
RIN	8.4	8.3	9.2	9	9.3

Figure 2. Yield, Purity, and Integrity of RNA Isolated from Mouse Lung and Kidney Using the RiboPure™ Kit

Mouse lung, previously stored in RNA*later* Solution, or frozen mouse kidney was homogenized in 1 mL of TRI Reagent per 100 mg tissue. RNA isolation was performed in duplicate (lung) or triplicate (kidney) using 1 mL aliquots of homogenate according to the RiboPure procedure. Yield and A_{260}/A_{280} ratios were determined using 2 µL of purified RNA on a NanoDrop® Spectrophotometer. The ratio of 28S to 18S rRNA and RIN values were obtained by analyzing 1 µL purified RNA using an RNA LabChip® Kit and the Agilent® 2100 bioanalyzer. See section *III.B. RNA Quality* on page 12 for further discussion of these metrics.

B. Materials and Reagents Included in the Kit

The RiboPure Kit contains reagents for 50 RNA isolations using ≤100 mg of tissue per isolation.

Amount	Component	Storage
50 mL	TRI Reagent	4°C
50	Filter Cartridges	room temp
100	Collection Tubes (2 mL)	room temp
60 mL	Wash Solution Concentrate Add 48 mL of 100% ethanol before use	room temp
11 mL	Elution Buffer	room temp

C. Materials Not Provided with the Kit

Required reagents

- 100% ethanol: ACS grade or higher quality
- 1-bromo-3-chloropropane (BCP), recommended (e.g., MRC Cat #BP 151), or chloroform ***without*** added isoamyl alcohol

Required equipment

- Apparatus for tissue grinding and homogenization
- 1.5 or 2 mL RNase-free microcentrifuge tubes, compatible with phenol/chloroform (e.g., P/N AM12400, AM12425)
- Microcentrifuge capable of at least 12,000 x g
- (Optional) The following supplies and equipment are needed to use vacuum pressure to pull solutions through the Filter Cartridges:
  – a vacuum manifold attached to vacuum pump
  – 50 sterile 5 mL syringe barrels to support the Filter Cartridges
- Pipettors and RNase-free pipet tips
- Vortex mixer

Optional materials and equipment for RNA analysis

- Spectrophotometer, e.g., the NanoDrop® 1000A Spectrophotometer, or materials for another RNA quantitation methodology, e.g., RiboGreen® RNA Quantitation Assay and Kit (Molecular Probes Inc.)
- Agilent® 2100 bioanalyzer, or reagents and apparatus for preparation and electrophoresis of agarose gels

D. Related Products

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-free Tubes & Tips see our product catalog	Ambion RNase-free tubes and tips are available in most commonly used sizes and styles. They are guaranteed RNase- and DNase-free. For more information, see our product catalog at www.invitrogen.com/ambion.
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.
Electrophoresis Reagents see our product catalog	We offer gel loading solutions, agaroses, acrylamide solutions, powdered gel buffer mixes, nuclease-free water, and RNA and DNA molecular weight markers for electrophoresis. For available products, see our product catalog at www.invitrogen.com/ambion.
RNA 6000 Ladder P/N AM7152	The RNA 6000 Ladder is a set of 6 RNA transcripts designed for use as reference standards with the RNA 6000 Lab Chip Kits and the Agilent 2100 bioanalyzer.
Millennium™ Markers and BrightStar® Biotinylated Millennium™ Markers P/N AM7150 and AM7170	Our 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.

II. RiboPure Kit Procedure

A. Experimental Setup

Tips for maximizing RNA yield from tissue samples

When working with tissue samples, it is important to obtain tissue quickly and to limit the time between obtaining tissue samples and inactivating RNases by one of the methods described in step 1a on page 7.

Choosing an effective and rapid method for tissue or cell disruption is also crucial. The most effective method is determined by the nature of the tissue, the storage method, and the size of the sample. For more information, go to www.invitrogen.com/site/us/en/home/support/technical-support.html.

RNase precautions

Lab bench and pipettors

Before working with RNA, it is always a good idea to clean the lab bench and pipettors with an RNase decontamination solution (e.g., Ambion RNase*Zap*® Solution).

Gloves and RNase-free technique

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

Use RNase-free pipette tips to handle Wash Solution and Elution Buffer, and avoid putting used tips into the kit reagents.

Use the Collection Tubes supplied with the kit; they have been tested for RNase contamination and are certified RNase-free.

Prepare Wash Solution

1. Add 48 mL of ACS grade 100% ethanol to the bottle labeled Wash Solution Concentrate and mix well.
2. Mark the label to indicate that the ethanol was added.
   The resulting mixture is called Wash Solution in the procedure.
   Store at room temperature for up to 1 month.

Inspect the Filter Cartridges

Briefly inspect the Filter Cartridges before use. Occasionally, the glass-fiber filters may become dislodged during shipping. If this is the case, gently push the filter down to the bottom of the cartridge using the wide end of an RNase-free pipet tip.

Procedure notes

- This procedure is designed for 5–100 mg tissue samples, 0.1–20 x 10^6 cultured cells, or up to 10 cm^2 of monolayer culture. For samples smaller than 5 mg or 0.1 x 10^6 cells, see our product catalog for available products at www.invitrogen.com/ambion.

- We recommend using BCP in step II.C.1 on page 9 because BCP is less toxic than chloroform and its use reduces the risk of contaminating RNA with DNA. Chloroform can be used for phase separation, but it should not contain isoamyl alcohol or any other additive.
- (Optional) An additional centrifugation step may be required for samples that have a high content of protein, fat, polysaccharide, or extracellular material, such as muscle, adipose tissue, or tuberous parts of plants. See step II.B.3 on page 9.
- (Optional) Solutions can be pulled through the Filter Cartridges with vacuum pressure rather than by centrifugation in section *II.D. Final RNA Purification* starting on page 10. Simply place the Filter Cartridges into sterile 5 mL syringe barrels mounted on a vacuum manifold.

B. Cell Disruption and Initial RNA Purification

1. Thoroughly homogenize samples in TRI Reagent

Follow the instructions for disruption of tissue samples in section 1a below. For cultured cells, follow section 1b on page 8.

1a. Homogenize tissue samples in 10–20 volumes of TRI Reagent

a. Tissue handling instructions

Handling fresh tissue: Obtain 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 phosphate-buffered saline (PBS), if desired, to eliminate some of the red blood cells.] 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).

As soon as possible after dissection, inactivate RNases by any one of the following treatments:

- Homogenize in TRI Reagent immediately (see *b. Tissue homogenization instructions* following).
- Freeze rapidly in liquid nitrogen (tissue pieces must be small enough to freeze in a few seconds).
- Submerge in a tissue storage buffer such as Ambion RNA*later* Solution.

Handling frozen tissue: Weigh frozen tissue, and if necessary, break it into pieces smaller than ~50 mg (keeping tissue completely frozen) and homogenize directly in TRI Reagent. Larger pieces of tissue, very hard or fibrous tissues, and tissues with a high RNase content must typically be ground to a powder in liquid nitrogen for maximum RNA yield.

Handling tissue that has been immersed in RNAlater Solution and then frozen: Thaw at room temperature before starting. Blot excess RNA*later* Solution from samples, and weigh them before following the instructions for homogenization in step b below.

b. Tissue homogenization instructions

Homogenize samples in 10–20 volumes TRI Reagent (e.g., 1 mL TRI Reagent per 50–100 mg tissue) using standard homogenization procedures. For most tissues, rotor-stator homogenizers are very effective.

1b. Homogenize cultured cells in 1 mL TRI Reagent per 10 cm^2 monolayer culture, or per 5 x 10^6 suspension culture cells

Do not wash cells before lysing with TRI Reagent as this may contribute to mRNA degradation.

Cells grown in monolayer: Remove media, add 1 mL of TRI Reagent per 10 cm^2 of culture dish area, and pass the mixture through a pipette several times to lyse cells and homogenize the sample. (Lyse directly in the culture dish.) Use the area of the culture dish, not the cell number, to determine the volume of TRI Reagent for lysis.

Cells grown in suspension: Pellet cells, remove media, then lyse in 1 mL of TRI Reagent per 5 x 10^6 animal, plant, or yeast cells by repeated pipetting or vortexing. Thorough disruption of some yeast cells may require the use of a homogenizer.

Frozen cell pellets: We recommend lysing cells in TRI Reagent and storing the frozen lysate, rather than freezing cell pellets. If you use frozen cell pellets, grind in liquid nitrogen in a mortar and pestle, then homogenize directly in TRI Reagent.

2. Incubate the homogenate for 5 min at room temp

Incubate homogenates from both tissue samples and cell cultures for 5 min at room temperature. This incubation allows nucleoprotein complexes to completely dissociate.

STOPPING POINT

Homogenized samples can be stored at –70°C for at least one month.

3. (Optional) Centrifuge at 12,000 x g for 10 min at 4°C and transfer the supernatant to a new tube

This optional centrifugation is required only to remove insoluble material from homogenates that contain high amounts of protein, fat, polysaccharide, or extracellular material, such as muscle, adipose tissue, and tuberous parts of plants. Centrifugation pellets extracellular membranes, polysaccharides, and high molecular weight DNA, leaving the RNA in the supernatant.

Adipose tissue samples: Lipids will form a layer on top; remove and discard this layer.

C. RNA Extraction

1. Add 100 µL of BCP to 1 mL of homogenate and mix well

a. Transfer 1 mL of homogenate to a labeled 1.5 mL microcentrifuge tube.

b. Add 100 µL of bromochloropropane (BCP) to the homogenate. Alternatively, use 200 µL of chloroform (***without*** isoamyl alcohol) in place of BCP.

c. Cap tubes tightly and vortex at maximum speed for 15 seconds.

2. Incubate for 5 min at room temp

Incubate the mixture at room temperature for 5 min.

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

Centrifuge at 12,000 x g for 10 min at 4°C to separate the mixture into a lower, red, organic phase (phenol-BCP phase); an interphase; and a colorless, upper, aqueous phase.

NOTE

Centrifugation at temperatures >8°C may cause some DNA to partition in the aqueous phase.

RNA remains in the aqueous phase while DNA and proteins are in the interphase and organic phase. The volume of the aqueous phase is typically about 60% of the volume of TRI Reagent used for homogenization.

4. Transfer 400 µL aqueous phase to a new 1.5 mL microcentrifuge tube.

Transfer 400 µL of the aqueous phase (top layer) to a new, labeled 1.5 mL microcentrifuge tube.

You can discard the tube after removing the aqueous phase, or the other phases can be processed for protein and/or DNA isolation. For more information, go to www.invitrogen.com/site/us/en/home/support/technical-support.html.

D. Final RNA Purification


NOTE

All centrifugations with Filter Cartridges should be done at 12,000 x g at room temperature.
Do not subject Filter Cartridges to relative centrifugal forces over 12,000 x g; higher forces may damage the filters.

1. Add 200 µL of 100% ethanol and mix immediately

a. Add 200 µL of 100% ethanol to 400 µL of aqueous phase from step C.4.

b. Vortex immediately at maximum speed for 5 seconds to avoid RNA precipitation.

2. Pass the sample through a Filter Cartridge

a. For each sample, place a Filter Cartridge in one of the Collection Tubes supplied.

b. Transfer the sample to a Filter Cartridge-Collection Tube assembly and close the lid.

c. Centrifuge the assembly at 12,000 x g for 30 seconds at room temperature or until all of the liquid is through the filter.

d. Discard the flow-through and return the Filter Cartridge to the same Collection Tube.
The RNA is now bound to the filter in the Filter Cartridge.

3. Wash the filter twice with 500 µL of Wash Solution

a. Apply 500 µL of Wash Solution to the Filter Cartridge-Collection Tube assembly, and close the lid.

b. Centrifuge for 30 seconds at room temperature or until all of the liquid is through the filter.

c. Discard the flow-through and return the Filter Cartridge to the same Collection Tube.

d. Repeat steps a–c for a second wash.

e. Centrifuge for 30 seconds at room temperature to remove residual Wash Solution.


NOTE

This step should be done using a centrifuge, not vacuum pressure.

f. Transfer the Filter Cartridge to a ***new*** Collection Tube.
Avoid contact of the Filter Cartridge with the flow-through when transferring to a new Collection Tube, to prevent carryover of ethanol in the eluted RNA and potential inhibition of downstream enzymatic reactions.

4. Elute RNA with 100 µL Elution Buffer

a. Add 100 µL of Elution Buffer to the filter column.

b. Incubate at room temperature for 2 min.

c. Centrifuge for 30 seconds to elute the RNA from the filter. The RNA will be in the eluate, in the Collection Tube.

d. (Optional) If desired, repeat steps a–c with a second aliquot of Elution Buffer, combining the second eluate with the first in the same Collection Tube; approximately 90% of the RNA is recovered during the first elution step.

NOTE

The exact volume of Elution Buffer used to recover the RNA is not critical and may be adjusted according to expected RNA yield and the desired RNA concentration. Using more Elution Buffer will maximize RNA recovery (up to a point), but the RNA will be more dilute.

e. Store the recovered RNA at 4°C or, for long term storage, at –20°C.

III. Analysis of the Recovered RNA

A. RNA Yield

Spectrophotometry

The concentration of an RNA solution can be determined by measuring its absorbance at 260 nm (A_{260}). Our scientists recommend using the NanoDrop 1000A Spectrophotometer because it is extremely quick and easy to use; just measure 1–2 µL of the RNA sample directly.

Alternatively, the RNA concentration can be determined by diluting an aliquot of the preparation in TE (10 mM Tris-HCl, pH 8, 1 mM EDTA) and reading the absorbance in a traditional spectrophotometer at 260 nm. To determine the RNA concentration in µg/mL, multiply the A_{260} by the dilution factor and the extinction coefficient (1 A_{260} = 40 µg RNA/mL).

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

Note 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.

B. RNA Quality

Microfluidics analysis

The Agilent 2100 bioanalyzer with Caliper's RNA LabChip® Kits provides better qualitative data than conventional gel analysis for characterizing RNA. When used with the 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 new metric developed by Agilent, the RIN analyzes information from both rRNA bands, as well as information contained outside the rRNA peaks (potential degradation products) to provide a fuller picture of RNA degradation states. Search for "RIN" at Agilent's website for information:

http://www.chem.agilent.com

Agarose gel electrophoresis

You can also assess the quality of your RNA sample by agarose gel electrophoresis. For more information, go to www.invitrogen.com/site/us/en/home/support/technical-support.html.

Spectrophotometry

An effective measure of RNA purity is the ratio of absorbance readings at 260 and 280 nm. The total RNA isolated with this kit should have an A_{260}/A_{280} ratio of 1.8–2.1. However, RNA with absorbance ratios outside of this range may still function well for qRT-PCR or other amplification-based downstream applications.

IV. Troubleshooting

A. Low Yield

Tissue or cells contain less RNA than expected

Expected yields of RNA vary widely between tissues. Tissues such as liver or kidney typically have higher RNA content and yields compared to tissues such as muscle, lung, or brain. See Figure 2 on page 3 for examples of yield data for mouse lung and kidney.

Poor tissue disruption

The most effective way to disrupt tissue is by grinding frozen tissues in liquid nitrogen and then homogenizing using a rotor-stator homogenizer. It is useful with very tough tissues to break the frozen tissue with a hammer before attempting to crush it in a mortar and pestle.

In some cases, it may be impossible to achieve complete disruption of the tissue. For example, due to a high content of connective tissue, breast tumor tissue is not amenable either to complete homogenization of fresh minced tissue or to thorough crushing of frozen tissue in liquid nitrogen. In such cases it may be advisable to go ahead with the isolation procedure after a reasonable effort at disruption, even if the tissue appears to still be mostly or partially intact.

For more information, go to www.invitrogen.com/site/us/en/home/support/technical-support.html..

Insufficient amount of TRI Reagent used

Use at least 10 volumes of TRI Reagent (i.e., ≥500 µL for a 50 mg tissue sample) for the initial extraction procedure. If the tissue is high in protein or fat, 20 volumes of TRI Reagent may be required.

For tissue stored in RNA*later* Solution, blot the RNA*later* Solution off as much as possible prior to disrupting in TRI Reagent.

B. Poor Quality or Purity

Partially degraded RNA

Poor tissue sample quality

It is important to limit the time between death and sample collection for the best yield of high quality RNA. It is extremely important to inactivate RNases as quickly as possible after sample collection to avoid RNA degradation. If tissue is obtained from an outside source, make sure that samples are either frozen or placed in RNA*later* Solution as soon as possible after dissection.

Frozen tissue thawed before immersion in TRI Reagent

It is essential that frozen tissue stays frozen until it is disrupted in TRI Reagent.

If the tissue is frozen in small pieces (i.e., <50 mg) and will be processed with an electronic rotor-stator homogenizer (Polytron type), it can be dropped directly into TRI Reagent and homogenized immediately. This shortcut generally works only for relatively soft tissues. Very hard or fibrous tissues, and tissues with a high RNase content, must typically be ground to a powder in liquid nitrogen for maximum RNA yield.

When powdering tissue with 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, mix it with TRI Reagent quickly before any of the powder can thaw.

Introduction of RNases during the procedure
Follow the best practices described in the section *RNase precautions* on page 6. Use RNase-free tubes, tips, and reagents.

DNA contamination

Insufficient amount of TRI Reagent was used for homogenization.
Use 1 mL TRI Reagent per 50–100 mg tissue, 5–10 x 10^6 cells, or 10 cm^2 culture dish area.

For cells grown in monolayer, use the area of the culture dish, not the cell number, to determine the appropriate volume of TRI Reagent needed.

Samples contained organic solvents, strong buffers, or alkaline solution.

Phase separation (step II.C.3 on page 9) was performed at temperatures above 8°C.
Some DNA may partition in the aqueous phase at temperatures >8°C.

Aqueous phase contaminated with interphase.
Avoid carryover when transferring the aqueous phase to a fresh tube in step II.C.4 on page 9.

Low A_{260}/A_{280} ratio (<1.6)

The volume of TRI Reagent used for sample homogenization was too low.
Use 1 mL TRI Reagent per 50–100 mg tissue, 5–10 x 10^6 cells, or 10 cm^2 culture dish area.

Nucleoproteins were incompletely dissociated from the RNA.
Allow the homogenate to stand at room temperature during step II.B.2 on page 8 for at least 5 min before further processing.

Aqueous phase was contaminated with the phenol phase.
Avoid carryover when transferring the aqueous phase to a fresh tube in step II.C.4 on page 9.

V. Appendix

A. Quality Control

Functional testing

Total RNA is isolated from mouse kidney using the RiboPure Kit according to this Protocol. RNA quality is analyzed on the Agilent 2100 bioanalyzer; the 28S rRNA:18S rRNA ratio is shown to be ≥1.2. DNA contamination is evaluated using qRT-PCR and is shown to be negligible.

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.

B. Safety Information

WARNING

GENERAL SAFETY. *Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.*

- *Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.*
- *Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.*

1. Chemical safety

WARNING

GENERAL CHEMICAL HANDLING. *To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:*

- *Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.*
- *Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).*
- *Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).*
- *Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.*
- *Handle chemical wastes in a fume hood.*
- *Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)*
- *After emptying a waste container, seal it with the cap provided.*
- *Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.*
- *Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.*
- ***IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.*

2. Biological hazard safety

WARNING

***Potential Biohazard.** Depending on the samples used on the instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.*

WARNING

***BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:*

In the U.S.:

- *U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: www.cdc.gov/biosafety*

- *Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html*
- *Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.*
- *Additional information about biohazard guidelines is available at: www.cdc.gov*

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

VI. Documentation and Support

A. Obtaining SDSs

Safety Data Sheets (SDSs) are available from:

www.invitrogen.com/sds

or

www.appliedbiosystems.com/sds

Note: For the SDSs of chemicals not distributed by Life Technologies, **contact the chemical manufacturer.**

B. Obtaining support

For the latest services and support information for all locations, go to:

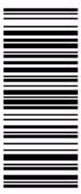
www.invitrogen.com

or

www.appliedbiosystems.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
- 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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