

PROTOCOL

life

ambion® | RNA
by *life* technologies™

PARIS™ Kit

life
technologies™

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

Information in this document is subject to change without notice.

APPLIED BIOSYSTEMS DISCLAIMS ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. TO THE FULLEST EXTENT ALLOWED BY LAW, IN NO EVENT SHALL APPLIED BIOSYSTEMS BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF, WHETHER OR NOT FORESEEABLE AND WHETHER OR NOT APPLIED BIOSYSTEMS IS ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

LIMITED USE LABEL LICENSE: RESEARCH USE ONLY

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

TRADEMARKS

The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

© 2011 Life Technologies Corporation. All rights reserved.

Part Number 1921M Rev. E
01/2011

PARIS™ Kit

(Part Number AM1921)

Protocol

I.	Introduction	1
	A. Product Description	
	B. Reagents Provided with the Kit and Storage	
	C. Required Materials Not Provided with the Kit	
	D. Related Products Available	
II.	PARIS Procedure	4
	A. Overview and Setup	
	B. Preparation of Lysate	
	C. RNA Isolation	
	D. (optional) Precipitate RNA with LiCl	
III.	Evaluating Yield and Quality	15
	A. Evaluating Yield and Quality of RNA Samples	
	B. Evaluating Yield and Quality of Protein Samples	
IV.	Troubleshooting	19
	A. Incomplete Cell Fractionation	
	B. Poor RNA Quality	
	C. Poor Protein Quality	
	D. Filter Clogging	
V.	Appendix	23
	A. Quality Control	
	B. Safety Information	
VI.	Documentation and Support	26
	A. Obtaining SDSs	
	B. Obtaining support	

I. Introduction



IMPORTANT

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

A. Product Description

PARIS™ is a rapid, phenol-free, small scale Protein And RNA Isolation System. It can be used to isolate total RNA and total protein directly from fresh cultured mammalian cells. Alternatively, the cells can be partitioned into nuclear and cytoplasmic fractions before undergoing the procedure. The PARIS Kit can also be used to obtain total RNA and total protein from most animal tissues that do not contain a high level of ribonucleases, and from cells or tissues that have been frozen in liquid nitrogen or stored in Ambion® RNAlater® or RNAlater-ICE Solution.



NOTE

Storage in RNAlater Solution will denature proteins, therefore total protein obtained from samples stored in RNAlater or RNAlater-ICE Solution will be competent for applications such as Western blotting or 2D gel electrophoresis, but will not be suitable for applications that require native protein.

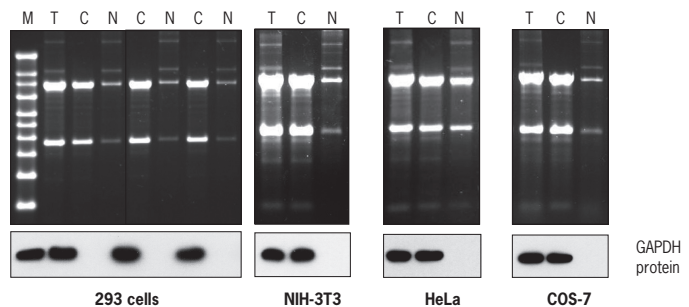
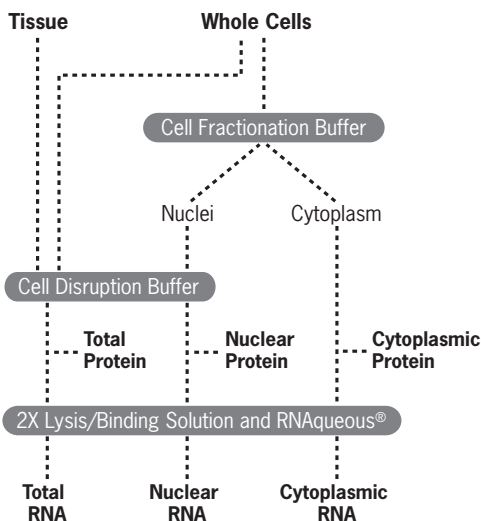


Figure 1. Comparison of Total, Cytoplasmic and Nuclear RNA and Protein from Different Cell Lines

RNA and protein were isolated from 10^6 cells using the PARIS procedure. To allow direct comparison between total (T), cytoplasmic (C) and nuclear (N) fractions, the same fraction (~5%) of each prep was analyzed by denaturing agarose gel electrophoresis or Western blot with anti-GAPDH antibodies.

The PARIS method (Figure 2) is based on differential lysis of plasma and nuclear cell membranes by nonionic detergents. Cells are either disrupted directly, or they are first separated into nuclear and cytoplasmic fractions. RNA is then isolated using Ambion RNAqueous® technology. The high quality RNA recovered can be used in any application, including RT-PCR, blot hybridization, in vitro translation, and cDNA synthesis. Total, nuclear and cytoplasmic protein fractions can be used directly for common applications such as enzymatic assays, immunoprecipitation, gel shift assays, two-dimensional gel electrophoresis, and Western blotting.

Figure 2. PARIS Procedure Flowchart



B. Reagents Provided with the Kit and Storage

This kit contains reagents to process 50 samples.

Amount	Component	Storage
30 mL	Cell Disruption Buffer	4°C
25 mL	Cell Fractionation Buffer	4°C
30 mL	2X Lysis/Binding Solution Add 415 µL 2-mercaptoethanol before use	4°C
40 mL	Wash Solution 1	4°C
80 mL	Wash Solution 2/3 Concentrate Add 64 mL 100% ethanol before use	4°C
4 mL	Lithium Chloride Precipitation Solution	4°C
50	Filter Cartridges	room temp
100	Collection Tubes	room temp
10 mL	Elution Solution	any temp*

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

C. Required Materials Not Provided with the Kit

Reagents

- 2-mercaptoethanol (14.3 M)
- 100% ethanol, ACS grade or better
- Phosphate buffered saline (PBS)

Equipment and supplies

- Heat block set to 95–100°C
- Microcentrifuge
- (optional) vacuum manifold: to pull solutions through the Filter Cartridges
- RNase-free microfuge tubes, pipettors and tips
- Tissue homogenizer (for solid tissue samples): motorized rotor-stator homogenizers work best, but for soft tissues and very small samples manual homogenizers may be sufficient – see section [II.B.2](#) starting on page [8](#) for more information on sample disruption.

D. Related Products Available

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.
RNA^{later}®-ICE Solution P/N AM7030, AM7031	RNA ^{later} -ICE Frozen Tissue Transition Solution is designed to make it easier to process frozen tissue samples for RNA isolation. Simply drop frozen tissues into RNA ^{later} -ICE Solution and store overnight at –20°C. Once tissues are treated they can be easily processed using standard RNA isolation procedures.
RNaseZap® Solution P/N AM9780, AM9782, AM9784	RNaseZap 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 RNaseZap Solution.
DNA-free™ Reagents P/N AM1906	DNase treatment and removal reagents. This product contains our 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.
TURBO™ DNase P/N AM2238, AM2239	TURBO™ DNase is a hyperactive DNase that was developed using a protein engineering approach to improve wild-type DNase I. These changes markedly increase the affinity of the protein for DNA. The result is a versatile enzyme with a 6-fold lower K _m for DNA, and an ability to maintain at least 50% of peak activity in solutions approaching 200 mM monovalent salt, even when the DNA concentration is in the nanomolar (nM) range.
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 .
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 .

II. PARIS Procedure

A. Overview and Setup

1. Overview of the PARIS procedure

The PARIS procedure is extremely flexible; it can be used to isolate both RNA and protein from 10^2 – 10^7 whole cells or from their nuclear and cytoplasmic fractions. Total RNA and protein can also be isolated from 0.5–75 mg samples of most animal tissues that do not contain high level of ribonucleases.

2. Before using the kit for the first time, complete the 2X Lysis/Binding Solution and Wash Solution 2/3

Add 415 μ L 2-mercaptoethanol to the 2X Lysis/Binding Solution, and mix well.

Place a check mark in the empty box on the label to indicate that the 2-mercaptoethanol has been added.

**NOTE**

The 2X Lysis/Binding Solution may solidify at 4°C. Before use, warm the solution at 37°C with occasional agitation for 5–10 min, or until it has completely redissolved. The 2X Lysis/Binding Solution can be stored at room temp for up to 1 month.

Add 64 mL 100% ethanol to Wash Solution 2/3, and mix well.

Use ACS grade 100% ethanol or better. Place a check mark in the empty box on the label to indicate that the ethanol has been added.

**NOTE**

A precipitate may form in the bottle over the next several days as excess EDTA precipitates from the solution. Simply leave these crystals in the bottle when removing Wash Solution 2/3 for use.

3. Do the following as you start the procedure:

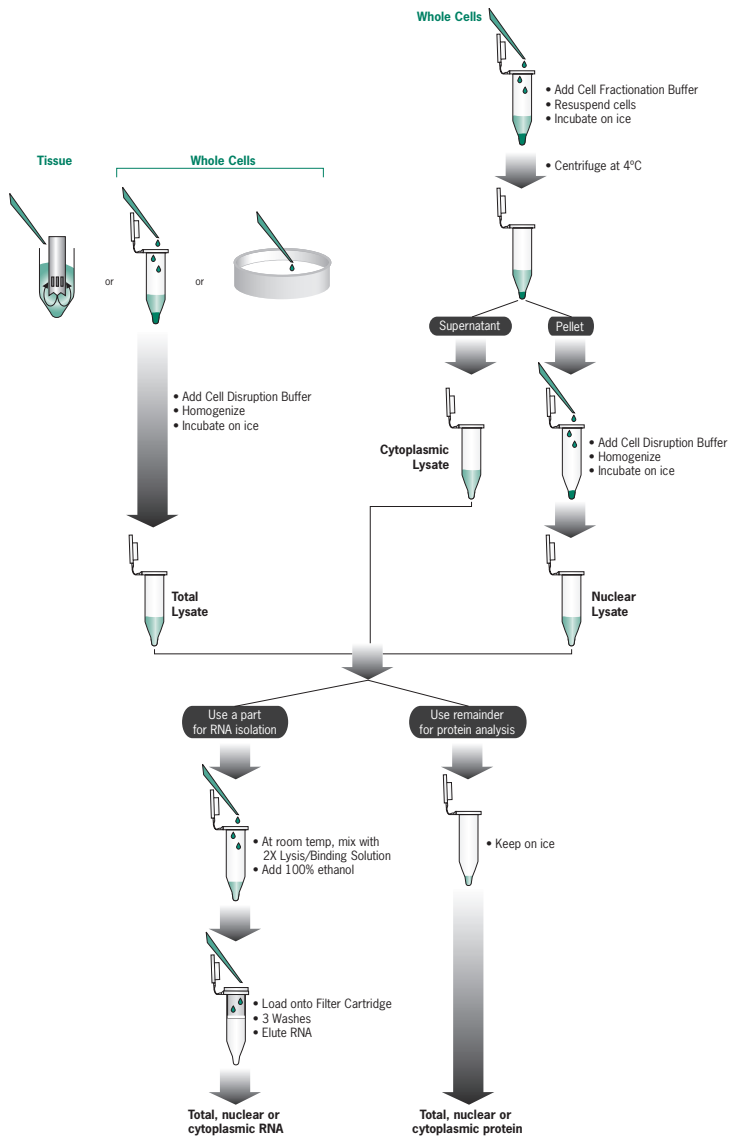
Clean the lab bench and equipment

Before working with RNA, it is always a good idea to clean the lab bench, pipettors, equipment used for tissue homogenization, etc., with an RNase decontamination solution (e.g. Ambion® RNaseZap Solution).

Bring kit components to the appropriate temperatures

- Place the bottles of Cell Disruption Buffer and/or Cell Fractionation Buffer on ice.
- Remove the 2X Lysis/Binding Solution and Wash Solution 1 from the refrigerator, and allow them to equilibrate to room temp. Inspect the solutions, and if they contain a precipitate, warm them to 37°C with occasional agitation to redissolve the precipitate.
- Heat an aliquot of Elution Solution (typically ~50–200 μ L per RNA isolation) in an RNase-free microfuge tube in a heat block set to 95–100°C.

Figure 3. PARIS Procedure Overview



Label and assemble the plasticware you will need for the procedure and aliquot the 2X Lysis/Binding Solution into tubes

- 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 pipette tip.
- Label the Filter Cartridges that will be needed for the experiment at hand. Then place them either into a Collection Tube (supplied) or into a 5 mL syringe barrel mounted on a vacuum manifold (depending on whether centrifugation or vacuum pressure will be used to draw solutions through the Filter Cartridges).
- Aliquot 2X Lysis/Binding Solution into labeled RNase-free tubes, at room temp, for use in step [C.1](#) on page 12.
Use a volume of 2X Lysis/Binding Solution equal to the sample volume that will be used for RNA isolation. If you are unsure what the sample volume will be, you can aliquot the 2X Lysis/Binding Solution immediately before use.

B. Preparation of Lysate

There are two sets of instructions for preparing sample lysate; if you are starting with fresh cultured mammalian cells, follow the appropriate instructions depending on your sample source, and goals.

- Fresh cultured mammalian cells:
 - To isolate RNA and/or protein from whole cells use the procedure in section [B.I](#) below.
 - To isolate RNA and/or protein from nuclear and cytoplasmic cell partitions use the procedure in section B.II starting on page 10.
- Animal tissue, or cultured mammalian cells that have been in storage (frozen, or stored in *RNAlater* or *RNAlater-ICE* solution):
Use the procedure in section [B.I](#) below to isolate RNA and protein from total cell lysates. Cell fractionation will not work for animal tissue samples or for stored cultured cells.

Tips for the best results with the PARIS procedure**Work quickly and keep samples cold during cell fractionation and/or cell disruption**

To avoid degradation of RNA by endogenous RNases, work quickly and keep everything on ice until the lysate is mixed with the 2X Lysis/Binding Solution at room temp. Complete the remainder of the RNA isolation procedure at room temp (otherwise components of the 2X Lysis/Binding Solution may precipitate).

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 Cell Disruption Buffer, Cell Fractionation Buffer, wash solutions, and the Elution Solution. Avoid putting used tips back into the kit reagents.

B.I. Preparation of Total Cell Lysate

This cell lysis procedure can be used with fresh or frozen cultured mammalian cells, or cells stored in *RNAlater* or *RNAlater-ICE* solution. It can also be used with fresh, frozen, or *RNAlater*-stored animal tissues that do not contain high levels of RNase. Following is a list of tissues known to be compatible or not compatible with the PARIS procedure:

Compatible	Not compatible
brain	spleen
liver	pancreas
kidney	
heart	

1. Collect 10^2 – 10^7 cells or 0.5–75 mg tissue, wash cells in cold PBS

In this procedure, sample size is limited by the amount of lysate that can be passed through a Filter Cartridge without clogging. These instructions are written to prepare the amount of lysate that can be processed on a single Filter Cartridge.

Cultured mammalian cells

Ideally cells in culture should be processed fresh (i.e. not frozen). If you need to store cells before RNA isolation, they can be stored in *RNAlater* solution, or they can be pelleted, snap-frozen in liquid nitrogen, and stored below -70°C .

Suspension cells: Count the cells, then pellet the volume of cell culture containing 10^2 – 10^7 cells at low speed, and discard the culture medium. Wash the cells by resuspending in ~ 1 mL PBS, and repelleting. Place the cells on ice.

***Adherent cells:* do one of the following**

- Aspirate and discard the culture medium, and rinse with PBS. Place the culture plate on ice.
- Trypsinize cells to detach them and count. Then inactivate the trypsin, pellet the cells, and discard the supernatant (following the method employed in your lab for the cell type). Wash the cells by gently resuspending in ~ 1 mL PBS, and pelleting at low speed. Place the cells on ice.

Tissue samples

For good yield of intact RNA, it is very important to obtain tissue quickly and to limit the time between obtaining tissue samples and inactivating RNases in step “c” below.

- a. 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 PBS if desired to eliminate some of the red blood cells.
- b. 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 RNAlater).
- c. Inactivate RNases by one of the following methods:
 - Drop the sample into RNAlater—tissue must be cut to ≤ 0.5 cm in at least one dimension.
 - Disrupt the sample in Cell Disruption Buffer as described in step 2. *Fresh (unfrozen) tissue* on page 9.
 - 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 below -70°C .

2. Disrupt samples in 100–600 μL ice-cold Cell Disruption Buffer

The exact volume of Cell Disruption Buffer is not critical. If your sample amount is near the suggested maximum (10^7 cells or 75 mg tissue), then use near the maximum recommended amount of Cell Disruption Buffer, and conversely, use a low end amount of buffer for relatively small samples. The Cell Disruption Buffer volume may also be adjusted according to the amount of lysate you want for protein analysis and its desired concentration. If required for specific applications, protease, phosphatase and/or RNase inhibitors can be added to an aliquot of Cell Disruption Buffer immediately before use.

Cultured cells

- a. Remove the PBS wash or the RNAlater, and add 100–500 μL of **ice-cold** Cell Disruption Buffer to the cells. Use at least 300 μL for $\geq 10^6$ cells. The cells will begin to lyse immediately upon exposure to the Cell Disruption Buffer. For adherent cells lysed directly in the culture plate, collect the lysate with a rubber spatula, and transfer it to a tube.
- b. Vortex or pipet vigorously to completely lyse the cells and to obtain a homogenous lysate. Keep samples cold during lysis. Large frozen cell pellets (i.e. containing more than about 10^7 cells) may need to be ground to a powder as described for frozen tissue samples to isolate high quality RNA.

**NOTE**

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

Solid tissue stored in Ambion RNAlater Solution, or transitioned to -20°C in RNAlater-ICE Solution.

Samples in RNAlater and RNAlater-ICE solution can usually be homogenized by following the instructions for fresh tissue (below). Extremely tough/fibrous tissues in RNAlater may need to be frozen and pulverized according to the instructions for frozen tissue in order to achieve good cell disruption.

Blot excess RNAlater from samples, and weigh them before following the instructions for fresh tissue below.

Fresh (unfrozen) tissue

- a. Blot tissue on absorbent paper to absorb excess moisture or RNAlater Solution. Measure or estimate the weight of the sample.
- b. Aliquot 100–600 μL (6–8 volumes per tissue mass) of **ice-cold** Cell Disruption Buffer into a homogenization vessel on ice.
- c. Thoroughly disrupt the tissue in Cell Disruption Buffer using a motorized rotor-stator homogenizer. A ground-glass homogenizer or a plastic pestle can be used for small pieces (less than ~ 10 mg) of soft tissue.

Frozen tissue, and extremely hard tissues

(Frozen tissue transitioned to -20°C in RNAlater-ICE Solution: process as for fresh tissue). Once the tissue has been removed from the -70°C freezer, it is important that it be processed immediately without even partial thawing. This is necessary because as cells thaw, ice crystals rupture both interior and exterior cellular compartments, releasing RNase.

- a. Blot tissue on absorbent paper to absorb excess moisture or RNAlater Solution. Measure or estimate the weight of the sample.
- b. Place 100–600 μL (6–8 volumes per tissue mass) of **ice-cold** Cell Disruption Buffer into a plastic weigh boat on ice.
We use a weigh boat because it is much easier to transfer the frozen powder to a weigh boat than to a tube of Cell Disruption Buffer.
- c. Grind frozen tissue to a powder with liquid nitrogen in a pre-chilled mortar and pestle sitting in a bed of dry ice.
- d. Using a pre-chilled metal spatula, scrape the powdered tissue into the Cell Disruption Buffer, and mix rapidly.
- e. Transfer the mixture to a vessel for homogenization and process the mixture to homogeneity. If available, use a motorized rotor-stator homogenizer (e.g. Polytron).

**IMPORTANT**

Keep the sample cold during disruption.

Just pick up the weigh boat and bend it to form a spout to transfer the material to a tube.

3. Split the lysate for RNA isolation and protein analysis

a. Immediately proceed to step [II.C.1](#) on page 12 with the portion of the lysate that will be used for RNA isolation.

Once the lysate is homogenized, immediately mix the portion that will be used for RNA isolation with an equal volume of 2X Lysis/Binding Solution at room temp (see step [II.C.1](#) on page 12). The high concentration of guanidinium thiocyanate in the 2X Lysis/Binding Solution will prevent RNA degradation by cellular ribonucleases.

b. Place the portion of lysate that will be used for protein analysis on ice for 5–10 min.

Incubate the remainder of the lysate on ice 5–10 min to ensure complete cell disruption before processing the sample for protein analysis. If desired, samples can be clarified by centrifugation at 4°C for 1–2 min at top speed in a microcentrifuge. Protein lysates may be very viscous, if this is the case, reduce viscosity by sonicating the lysate, or by passing it through a syringe needle several times.

B.II. Preparation of Separate Nuclear and Cytoplasmic Lysate



IMPORTANT

Only actively growing cultured mammalian cells can be used in this procedure. Freezing/thawing of cells, or storage in RNAlater or RNAlater-ICE solution will impair the cell fractionation process. Also, we do NOT recommend trying to use solid tissue samples in this procedure.

1. Collect up to 10⁷ fresh cultured cells, wash once in PBS, and place washed cells on ice

Use at most 10⁷ cells per experiment. The minimum number of cells that can be used in this procedure is limited only by your ability to see the nuclear fraction so that you can withdraw the cytoplasmic fraction in step [5](#) below. For example, with HeLa cells, the minimum practical number of cells to use is 5 x 10⁴–1 x 10⁵.

a. Trypsinize adherent cells, inactivate the trypsin, count the cells, and place them in a microfuge tube.

For cells grown in suspension, simply count the cells, and place them in a microfuge tube.

b. Pellet cells at low speed, discard the culture medium, and wash the cell pellet once in PBS. Keep cells on ice.

2. Resuspend cells in 100–500 µL ice-cold Cell Fractionation Buffer

The exact volume of Cell Fractionation Buffer is not critical, but we do recommend using at least 300 µL Cell Fractionation Buffer for 10⁶ cells or more. The volume of Cell Fractionation Buffer can be chosen according to the amount of lysate you want for protein analysis, and the

desired protein concentration of the lysate. If required for specific applications, protease, phosphatase and/or RNase inhibitors can be added to an aliquot of Cell Fractionation Buffer immediately before use.

- a. Remove the PBS wash, and add 100–500 μL of *ice-cold* Cell Fractionation Buffer to the cells.
- b. Loosen the cell pellet by flicking the tube. *Gently* resuspend the cells by vortexing or pipetting. Vigorous handling at this step can result in partial nuclear lysis and contamination of the cytoplasmic fraction with nuclear components.

3. Incubate on ice 5–10 min

The suspension should clear rapidly indicating that lysis of the plasma membrane has occurred. Incomplete lysis will result in contamination of the nuclear fraction with cytoplasmic components.

4. Centrifuge samples 1–5 min at 4°C and 500 x g

This centrifugation separates the nuclear and cytoplasmic cell fractions. It is important to use a low speed centrifugation to keep the nuclei intact.

5. Carefully aspirate the cytoplasmic fraction away from the nuclear pellet

While carefully avoiding the nuclear pellet, collect the supernatant containing the cytoplasmic fraction with a micropipettor (we typically use a 200 μL pipet tip).

Cytoplasmic fraction: Put the supernatant in a fresh RNase-free tube if you want to isolate cytoplasmic RNA and protein. Keep on ice, and proceed immediately to step 8 below.

Nuclear fraction: The pellet contains the nuclear fraction, after removing the supernatant, proceed through the following steps.

6. (optional) Wash the nuclear pellet in ice-cold Cell Fractionation Buffer

This step may be necessary if the nuclear pellet becomes loose during removal of the supernatant. Washing the pellet will prevent contamination of the nuclear fraction with components of the cytoplasmic fraction.

- a. Add the volume of *ice-cold* Cell Fractionation Buffer used in step 2 to the nuclear pellet.
- b. Gently flick the tube and resuspend the nuclei 1 min at 4°C and 500 x g. Aspirate and discard the supernatant.

7. Lyse nuclear pellet in Cell Disruption Buffer

a. Add 100–500 μL of *ice-cold* Cell Disruption Buffer to the nuclear pellet. To keep cytoplasmic and nuclear samples parallel, you may want to use a volume of Cell Disruption Buffer equal to the volume of Cell Fractionation Buffer used in step 2.

b. Vortex or pipet vigorously to lyse the nuclei, and continue disruption until the lysate is homogenous. Keep on ice.

8. Split the sample for RNA isolation and protein analysis

a. Immediately proceed to step [II.C.1](#) on page 12 with the portion of the sample that will be used for RNA isolation.

Mix the portion of the cytoplasmic fraction and/or the nuclear lysate that will be used for RNA isolation with an equal volume of 2X Lysis/Binding Solution at room temperature (see step [II.C.1](#) on page 12). The high concentration of guanidinium thiocyanate in the 2X Lysis/Binding Solution will prevent RNA degradation by cellular ribonucleases.

b. Protein samples:

Nuclear fraction: Incubate nuclear fraction protein samples on ice 5–10 min to ensure complete cell disruption before processing the sample for protein analysis. Nuclear fraction protein lysates may be very viscous, if this is the case, reduce viscosity by sonicating the lysate, or by passing it through a syringe needle several times.

Cytoplasmic fraction: Cytoplasmic fraction protein samples do not require another incubation on ice.

Both fractions: If desired, protein samples can be clarified by centrifugation at 4°C for 1–2 min at top speed in a microcentrifuge.

Information on protein quantitation and quality evaluation is provided in section [III.B](#) starting on page 16.

C. RNA Isolation



IMPORTANT

This procedure should be done at room temperature because the 2X Lysis/Binding Solution may precipitate at colder temperatures.

All centrifugations should be at RCF 10,000–15,000 Xg (typically 10,000–14,000 rpm). Spinning with more force than this may damage the filters in the Filter Cartridges.

1. Mix the lysate with an equal volume of 2X Lysis/Binding Solution

Record the volume of the lysate used for RNA isolation; this **sample volume** will equal the volume of 100% ethanol to use in step [3](#).

Transfer the lysate that will be used for RNA isolation to a tube containing an equal amount of 2X Lysis/Binding Solution at room temp. Immediately mix gently but thoroughly by pipetting 3–4 times, or by inverting the tube several times.

2. If necessary, reduce viscosity and remove debris from the sample

The following precautions can help avoid clogging of the Filter Cartridge later in the procedure.

- Lysate should be somewhat viscous, but if a sample is so viscous that it is difficult to pipet, reduce the viscosity by sonicating the lysate, or by passing it through a syringe needle several times.
- If a sample contains visible debris, remove it by spinning in a microcentrifuge for 1 min at 10,000 x g.

3. Add 1 “sample volume” of 100% ethanol to the mixture

Add a volume of ACS grade 100% ethanol equal to the *sample volume* from step 1 (volume *before* adding the 2X Lysis/Binding Solution). Mix gently but thoroughly by pipetting 3–4 times, or by inverting the tube several times.

4. Draw the sample mixture through a Filter Cartridge

a. Apply the sample mixture to a Filter Cartridge assembled in either a Collection Tube (supplied) or a 5 mL syringe barrel on a vacuum manifold.

The maximum volume that can be applied at one time is ~700 μ L.

If you are using a vacuum manifold, apply the vacuum to draw the lysate through the filter and proceed to step 5. Leave the vacuum on to pull the washes through in subsequent steps.

b. Centrifuge for ~0.5–1 min or until the lysate/ethanol mixture is through the filter.

c. Discard the flow-through and reuse the Collection Tube for the washing steps.

d. If the sample volume exceeds 700 μ L, apply the remaining mixture after discarding the flow-through from the collection tube. Generally up to ~2 mL of sample mixture can be passed through the filter without clogging or exceeding its RNA binding capacity.

5. Wash once with 700 μ L Wash Solution 1

Apply 700 μ L Wash Solution 1 to the Filter Cartridge. Centrifuge for ~15 sec to 1 min or until all of the solution is through the filter. Discard the flow-through, and reuse the tube for subsequent washes.

If you are using a vacuum manifold, simply apply the wash solution, and the vacuum pressure will draw it through the filter.

6. Wash with 2 x 500 μ L Wash Solution 2/3

a. Apply 500 μ L Wash Solution 2/3. Draw it through the filter as in the previous step.

b. Repeat with a second 500 μ L aliquot of Wash Solution 2/3.

c. After discarding the wash solution, continue centrifugation, or leave on the vacuum manifold for ~10–30 sec to remove the last traces of Wash Solution 2/3.

7. Elute RNA with 40–60 μ L of ~95°C Elution Solution

a. Put the Filter Cartridge into a fresh Collection Tube (supplied).

b. Apply Elution Solution preheated to ~95–100°C to the center of the filter.

The exact volume of Elution Solution used is not critical, but it is important to elute the RNA with 2 sequential aliquots of Elution Solution, and to use hot Elution Solution (95–100°C). The minimum practical volume of Elution Solution to use is 50 μ L, applied as sequential aliquots of 40 μ L and 10 μ L.

c. Recover the RNA by centrifugation for ~30 sec.

**8. Elute with a second
10–60 µL aliquot of ~95°C
Elution Solution**

Add a second aliquot of hot Elution Solution to the center of the filter and re-spin for ~30 sec.

Typically, this second elution is collected into the same tube as the first elution.

9. Store your RNA at –80°C

Ideally RNA samples should be stored at –80°C and kept on ice when in use.

For many applications, the RNA is ready to use at this point. If the RNA will be used in RT-PCR, you may want to remove trace DNA contamination by treating the RNA with DNase.

Information on RNA quantitation and quality evaluation is provided in section [III.A](#) starting on page 15.

D. (optional) Precipitate RNA with LiCl

Include this precipitation to concentrate the RNA and/or to remove carbohydrates and gross DNA contamination. Check the concentration of your RNA before starting this procedure, because it should be at least 200 ng/µL to assure efficient precipitation. Also, LiCl precipitation may not quantitatively precipitate RNAs smaller than 300 nt.

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

III. Evaluating Yield and Quality

A. Evaluating Yield and Quality of RNA Samples

1. RNA concentration

The concentration of RNA can be determined by diluting an aliquot of the preparation, and reading the absorbance in a spectrophotometer at 260 nm. Include a measurement at 280 nm as well to get a rough idea of RNA purity (see section [A.3](#) below). An A_{260} of 1 is equivalent to 40 μg RNA/mL. Find the concentration in $\mu\text{g}/\text{mL}$ by multiplying the A_{260} by the dilution factor and the extinction coefficient.

$$(1 A_{260} = 40 \mu\text{g RNA/mL})$$

$$A_{260} \times \text{dilution factor} \times 40 = \mu\text{g RNA/mL}$$

- The buffer used for dilution does not need to be RNase-free (unless you want to recover the RNA), since slight degradation of the RNA will not significantly affect its absorbance.
- 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.

2. RNA yield

RNA yield will widely vary according to the type and amount of sample, but will typically fall in the range of $\sim 1 \mu\text{g}$ per 10^5 cells in culture, or 1–10 μg per mg tissue. Researchers accustomed to working with tissues such as liver or kidney where RNA is plentiful may have unrealistically high expectations of RNA yields from tissues such as muscle, lung, brain or breast tumor tissue.

The ratio of nuclear to cytoplasmic RNA is cell line dependent; it generally falls between 0.5 and 2. When RNA is isolated from nuclear and cytoplasmic cell fractions, the sum of the mass of nuclear RNA and cytoplasmic RNA preps should be roughly equivalent to the amount of RNA isolated from the same number of whole cells.

3. RNA quality and downstream applications

RNA purity

The ratio of A_{260} to A_{280} values is a measure of RNA purity, pure RNA will fall in the range of 1.8 to 2.1. Even if an RNA prep has an $A_{260}:A_{280}$ ratio outside of this range, it may function well in common applications such as Northern blotting, and RT-PCR.

Overall RNA quality

The overall quality of an RNA preparation can be assessed by electrophoresis on a denaturing formaldehyde or glyoxal-based agarose gel (see [Figure 1](#)). High quality RNA will have fairly compact rRNA bands, and the 28S rRNA band will be about twice as intense as the 18S band. RNA degradation will cause smearing of the rRNA bands. Glyoxal-based gels eliminate the safety issues associated with formaldehyde-based gel systems, and they may produce sharper bands. Either

way denaturing agarose gel analysis will also give some information about RNA yield and DNA contamination. Yield can be estimated by comparing the staining of rRNA with a sample of known concentration or with a molecular weight marker. DNA, if present, will be evident as a high molecular weight smear or band migrating more slowly than the 28S rRNA band.

The integrity of the RNA can also be evaluated by capillary electrophoresis using an Agilent 2100 bioanalyzer. A 28S:18S rRNA peak ratio at or near 2:1 indicates that the RNA is intact.

Separation of nuclei and cytoplasm

Efficient nuclear/cytoplasmic fractionation can be quickly assessed by denaturing agarose gel analysis (see Figure 1). Bands corresponding to precursor rRNA should be equivalent in the total and nuclear RNA fraction. The 18S and 28S rRNA bands will be less intense in the nuclear RNA fraction than in either total RNA or cytoplasmic fraction RNA. In some cell lines, for example 293 and COS-7 cells, this difference will be dramatic, but in other cells lines such as HeLa cells, the rRNA bands are only slightly less intense in nuclear RNA than in total or cytoplasmic fraction RNA.

RT-PCR and trace genomic DNA contamination

RNA isolated with the PARIS Kit can be used directly for most common applications. DNase 1 treatment, however, is recommended for RNA that will be used for RT-PCR. It is especially important when using primers that do not flank introns, or for genes that have processed pseudogenes, because the RT-PCR products from RNA and contaminating DNA cannot be distinguished by size in these cases. The DNase 1 and divalent cations introduced by the DNase 1 Buffer must be removed from the RNA sample prior to RT-PCR. Divalent cations could degrade RNA at temperatures typically used for RNA denaturation prior to reverse transcription and residual DNase 1 could degrade DNA made in the RT-PCR. Ambion® DNA-free™ DNase Treatment and Removal Reagents were designed for this application.

B. Evaluating Yield and Quality of Protein Samples

1. Storage or protein samples

Protein samples can be stored at -20°C up to 1 week but should be snap frozen in liquid nitrogen and stored at or below -70°C for longer storage, or for use in functional assays. Occasionally residual nucleic acids in the sample may form a white precipitate upon thawing. This precipitate can be removed by centrifugation at 4°C for 1–2 min at top speed in a microcentrifuge without affecting the protein in the sample.

2. Protein concentration

The concentration of protein samples can be assessed using any standard protein quantitation procedure, including the BCA method, the Bradford assay, the Lowry assay or other commercially available protein quantitation assays. The protein concentration can be manipulated during the PARIS procedure if desired, by varying the volume of Cell Disruption Buffer or Cell Fractionation Buffer used for cell fractionation and cell, tissue or nuclei disruption.

For analyses such as gel shift assays or enzymatic assays, protein is usually concentrated enough to be diluted in the reaction buffer. If necessary, however, samples can be concentrated using standard procedures such as acetone or TCA precipitation, vacuum drying, or centrifugal concentrators.

3. Protein yield

The yield of protein is dependent on the tissue and cell line used in the procedure, but in general expect ~200–400 µg protein or more per 10⁶ cells. Typically the protein yield from cytoplasmic cell fractions will be 2–4 times higher than from nuclear cell fractions depending on the cell type used. The sum of nuclear and cytoplasmic protein yields should be roughly equivalent to the amount of protein isolated from the same number of whole cells.

4. Protein quality and downstream applications

Total, nuclear, and cytoplasmic protein samples can be used directly for applications such as immunoprecipitation and Western blotting that can tolerate the salt and detergent carried over from the PARIS procedure. For use in applications such as enzymatic assays, gel shift assays, and two-dimensional gel electrophoresis, it may be necessary to further purify protein samples.

Removing reagents carried over from the PARIS procedure

If salts and/or detergents present in the Cell Disruption Buffer or Cell Fractionation Buffer interfere with your experiments, they can be removed by dialysis or by protein purification on gel-filtration columns.

For 2D gel electrophoresis, treat samples with DNase

Protein samples from whole cells and from nuclear cell fractions contain all of the cellular genomic DNA and can be very viscous. For two-dimensional gel electrophoresis analysis, we recommend treating the samples with DNase I. Ambion TURBO DNase, which is extremely tolerant of salt and detergent, can be added directly to the Cell Disruption Buffer or the Cell Fractionation Buffer to remove genomic DNA. Alternatively, protein samples can be treated to remove salts and detergents so that they can be digested with ordinary DNase I.

Separation of nuclei and cytoplasm

The effectiveness of cell fractionation can be easily checked by Western blotting with antibodies for proteins found predominantly in the nucleus or cytoplasm of cells such as Ambion anti-GAPDH antibody (see Figure 1 on page 1—GAPDH is a cytoplasmic protein).

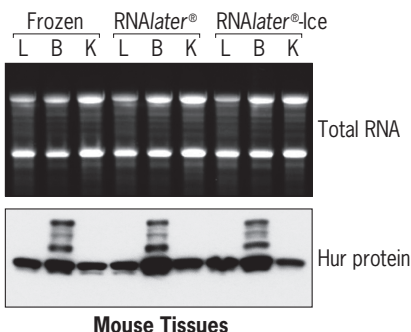


Figure 4. RNA and protein from stored tissues

RNA and protein was obtained from ~30 mg of mouse liver (L), brain (B) or kidney (K) using the PARIS Kit 5 days after tissue collection. Samples were either kept frozen at -80°C, stored in RNAlater at 4°C or snap frozen and then stored in RNAlater-ICE at -20°C. Samples were analyzed by denaturing agarose gel electrophoresis or Western blot with anti-Hur antibodies.

IV. Troubleshooting

A. Incomplete Cell Fractionation

Specific problems discussed below will result in varying degrees of cross contamination between the nuclear and cytoplasmic fractions. The most obvious cause of ineffective cell fractionation is mixture of the fractions while collecting the cytoplasmic supernatant in step [II.B.5](#) on page 11. Be careful to avoid touching the nuclear pellet while aspirating the cytoplasmic fraction. If the nuclear pellet begins to disperse, aspirate as much supernatant as possible, then recentrifuge what is left in the tube at 4°C for 10–30 sec at 500 x g, and collect the remainder of the cytoplasmic fraction. To eliminate any contamination of the nuclear fraction by cytoplasmic components, include the extra washing step described in step [II.B.6](#).

1. Low cytoplasmic yield

Incomplete lysis of the plasma membrane in the Cell Fractionation Buffer will result in only partial recovery of the cytoplasm and contamination of the nuclear fraction with cytoplasmic components. For good plasma membrane lysis be sure to gently but thoroughly resuspend cell pellets. Lysis should occur almost immediately causing a rapid clearing of the suspension.

Lysis can be assessed by adding Trypan Blue solution to an aliquot of the mixture, and observing the cells under the microscope. Trypan Blue stains the nuclei of lysed cells but is excluded from intact cells.

For complete plasma membrane lysis of some cell types, in step [II.B.2](#) on page 10, the incubation time on ice and the volume of Cell Fractionation Buffer may need to be increased.

2. Low nuclear yield

a. Nuclear leakage

Excessive membrane lysis in Cell Fractionation Buffer or harsh cell resuspension will lyse some or all of the nuclei, resulting in only partial recovery of the nuclear fraction and contamination of the cytoplasmic fraction with nuclear components.

After adding the Cell Fractionation Buffer, disperse the cell pellet by gently flicking the tube. Also, avoid aspirating cells into the pipettor when mixing cells with the buffer. If the suspension become viscous or if lysis of nuclei is observed under the microscope, reduce the incubation time on ice, and increase the volume of Cell Fractionation Buffer.

b. Inadequate collection of the nuclei

According to the number and type of cell used, the centrifugal force and time used to separate cytoplasm from nuclei in step [II.B.4](#) on page 11 may need to be adjusted. You can check for complete removal of the nuclei from the supernatant by observing an aliquot of the cytoplasmic fraction under the microscope.

Separating the fractions is more difficult when a very small number of cells are fractionated, because the nuclear pellet may not be visible at the bottom of the microfuge tube.

B. Poor RNA Quality

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

1. RNA looks degraded on a gel**a. Ribosomal RNA is overloaded**

Running more than about 5 µg of RNA in a single lane may cause smearing and/or smearing of the rRNA bands. Remember, rRNA comprises about 80% of total RNA, so loading 5 µg of total RNA in a single gel lane will give almost 2 µg of nucleic acid in each rRNA band.

b. Samples are incompletely denatured

Most RNA forms extensive secondary structure via intramolecular base pairing, and this prevents it from migrating strictly according to its size. To completely denature RNA, the sample must be both adequately diluted in the gel loading dye, and incubated for enough time at an elevated temperature.

- with Ambion NorthernMax® Formaldehyde Load Dye (P/N AM8552): Mix at least 3 volumes Formaldehyde Load Dye with the RNA, and then incubate at 65°C for ≥15 min.
- with Ambion NorthernMax Glyoxal Load Dye (P/N AM8551): use equal volumes RNA and Glyoxal Load Dye, and incubate 30 min at 50°C.

Also, 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 being analyzed. RNA molecular weight markers, an RNA sample known to be intact, or both, can be used for this purpose.

c. 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. For long runs (>3 hr) the buffer may need to be circulated to avoid the formation of pH gradients in the gel.

2. RNA is degraded

a. Improper handling of sample

It is extremely important to collect, process and homogenize samples as quickly as possible to avoid RNA degradation by endogenous ribonucleases. It is also very important to keep samples, Cell Disruption Buffer, and Cell Fraction Buffer as cold as possible (i.e. on ice). If desired, RNase inhibitors such as SUPERase•In™ can be added at 100–1000 U/mL to these solutions just before use.

Immediately after homogenization, lysate should be mixed with the 2X Lysis/Binding Solution at room temperature. This solution contains guanidinium thiocyanate, a strong chaotropic denaturant which rapidly inactivates RNases.

b. Exogenous RNase contamination

Once the lysate is bound to the filter in the Filter Cartridge, and the Lysis/Binding Solution has been washed away, all the typical precautions against RNase contamination should be rigorously observed. For example wear gloves at all times, and change them frequently to avoid introducing “finger RNases”. Keep the bags containing the Collection Tubes, and the solution bottles closed when they are not in use to avoid contamination with dust. Any tubes or solutions not supplied with the kit, which will contact the RNA, should be bought or prepared so that they are free from RNases.

C. Poor Protein Quality

1. No or low protein activity

a. Improper handling of sample

It is important to keep samples cold during the procedure. Keep samples on ice between each step and centrifuge at 4°C when required. Also, complete the procedure as quickly as possible to get the highest quality protein (and RNA).

To store protein aliquots, snap-freeze them in liquid nitrogen and store below –70°C for use in functional assays.

b. Protein degradation or modification

If required, protease inhibitors, phosphatase inhibitors and anti-oxidants can be added to the Cell Disruption Buffer and/or the Cell Fractionation Buffer just before use. These reagents will not adversely affect subsequent RNA isolation from samples.

2. Low protein concentration

a. Incomplete sample disruption

Incomplete sample homogenization will result in only partial recovery of protein. Some tissues may be more difficult to disrupt than others, especially if they are frozen. Increase the duration of mechanical homogenization and subsequent incubation on ice if required.

When including a centrifugation step to clarify the protein lysate, be sure that the sample is thoroughly homogenized before centrifugation.

b. Volume of Cell Disruption Buffer or Cell Fractionation Buffer was too high

As little as 100–300 μL of Cell Disruption Buffer or Cell Fractionation Buffer can be used in the PARIS procedure to maximize protein concentration. For most cells, we recommend using at least 300 μL of buffer when isolating RNA and protein from $\geq 10^6$ cells. The buffer volume should be sufficient to resuspend the cells by gentle vortexing.

Using too little Cell Fractionation Buffer may compromise cell fractionation because more force will be needed to resuspend the cells, and this could damage nuclear membranes. If too little Cell Disruption Buffer is used, the sample may be too viscous to process properly, thus compromising RNA yield and quality.

D. Filter Clogging

Difficulty moving the samples or wash solutions through the filter in the Filter Cartridge is most often encountered when processing relatively large amounts of solid tissue, and is more pronounced with certain types of tissue (e.g. liver and kidney).

1. Homogenize samples more thoroughly

Filter clogging can often be reduced by diluting and more thoroughly homogenizing the sample. Add more 2X Lysis/Binding Solution and an equal volume of Cell Disruption Buffer or Cell Fractionation Buffer and homogenize again by pipetting or vortexing, or by inverting the tube several times.

2. Reduce sample viscosity

It may help to reduce the viscosity of the lysate by sonication or by passing it through a syringe needle to shear DNA.

3. Do a clarifying spin

If filter clogging is still a problem after trying the previous suggestions, use a clarifying centrifugation just *before* adding ethanol in step [II.C.3](#) on page 13. This will remove debris and insoluble material. Spin the lysate at 10,000 $\times g$ for 1 min, and remove the clear supernatant to a fresh tube before adding the ethanol.

It is important to do this clarifying spin before adding the ethanol to avoid loss of RNA at this step. Avoid clarifying the lysate at too high a centrifugal force or for too long a time, as this may also lead to loss of RNA.

V. Appendix

A. Quality Control

Functional testing

Following the procedure in Section II, obtain a yield of >10 µg total RNA and >200 µg total protein per 10⁶ HeLa cells.

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



1921ME

Headquarters

5791 Van Allen Way | Carlsbad, CA 92008 USA
Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

For support visit www.appliedbiosystems.com/support

www.lifetechnologies.com

