Invitrogen™
life technologies

Instruction Manual

# PureLink™ 96 Total RNA Purification Kit

## For high-throughput purification of total RNA

**Catalog no. 12173-011**

**Version C**
**20** April 2005
*25-0640*

# Table of Contents

# Kit Contents and Storage

## Shipping and Storage

All components of the PureLink™ 96 RNA Purification Kit are shipped at room temperature. Upon receipt, store all components at room temperature.

## Contents

The components included in the PureLink™ 96 RNA Purification Kit are listed below. Sufficient reagents are included in the kit to perform 384 (4 x 96) isolations.

| Item | Amount |
|---|---|
| PureLink™ RNA Lysis Solution (1X) | 150 ml |
| PureLink™ Wash Buffer I (1X) | 450 ml |
| PureLink™ Wash Buffer II (5X) | 175 ml |
| RNase-free Water | 75 ml |
| PureLink™ 96-Well Filter Plates | 4 Plates |
| PureLink™ 96-Well Receiver Plates | 4 Plates |

## Product Qualification

The PureLink™ 96 RNA Purification Kit is functionally qualified by isolating total RNA from $10^5$ HeLa cells as described in this manual and must produce the following results:

- $OD_{260/280}$ between 1.9 and 2.1
- Intact RNA as determined by visual inspection on an agarose gel

In addition, each kit component is sterile and free of ribonuclease contamination, and is lot qualified for optimal performance.

# Accessory Products

## Additional Products

The following products are also available from Invitrogen. For more details on these products, visit our Web site at www.invitrogen.com or contact Technical Service (page 18).

| Product | Quantity | Catalog no. |
|---|---|---|
| **PureLink™ 96 RNA Components Available Separately** | | |
| PureLink™ 96 RNA Lysis Buffer | 750 ml | 12173-022 |
| PureLink™ 96 RNA Wash Buffer I (1X) | 5 x 1 L | 12173-032 |
| PureLink™ 96 RNA Wash Buffer II (5X) | 2 x 1 L | 12173-033 |
| PureLink™ 96 RNA Filter Plates | 50 pack | 12173-035 |
| PureLink™ 96 Receiver Plates (deep-well) | 50 pack | 12193-025 |
| **Reagents for RT-PCR** | | |
| SuperScript™ One-Step RT-PCR System with Platinum® *Taq* DNA Polymerase | 100 reactions | 10928-042 |
| Platinum® Quantitative RT-PCR ThermoScript™ One-Step System | 100 reactions | 11731-015 |
| LUX™ Fluorogenic Primer Set, FAM-labeled | 50 nmol or 200 nmol | Design and order LUX™ Primer Sets at www.invitrogen.com /lux |
| LUX™ Fluorogenic Primer Set, JOE-labeled | 50 nmol or 200 nmol | |
| **Reagents** | | |
| RNase *AWAY*® | 250 ml | 10328-011 |
| DNase I | 20,000 units | 18047-019 |
| DNase I, Amplification Grade | 100 units | 18068-015 |
| 0.16-1.77 Kb RNA Ladder | 75 µg | 15623-010 |
| 0.24-9.5 Kb RNA Ladder | 75 µg | 15620-016 |
| UltraPure™ DEPC-treated Water | 1 L | 750023 |
| UltraPure™ DNase/RNase-Free Distilled Water | 500 ml | 10977-015 |

# Introduction

# Overview

## Introduction

The PureLink™ 96 RNA Purification Kit allows high-throughput isolation of total RNA from 96 different cell samples. The kit is designed to isolate total RNA from various sources such as bacteria, yeast, plant, viruses, and mammalian cells and tissues.

The PureLink™ 96 RNA Purification System is designed for use with a vacuum manifold or a centrifuge and is compatible with most automated liquid handling workstations.

## System Overview

Cells are lysed using the RNA Lysis Solution containing guanidine isothiocyanate, a chaotrope capable of protecting the RNA from endogenous RNases. Ethanol is added to the lysate and the lysate is processed through the 96-well Filter Plate. RNA binds to the silica-based membrane in the Filter Plate and impurities are removed by thorough washing with Wash Buffers. The RNA is eluted in RNase free/DEPC-treated water.

## Advantages

Using the PureLink™ 96 RNA Purification Kit to isolate total RNA offers the following advantages:

- Higher yields and purity as compared to other commercially available RNA purification systems
- Designed to isolate total RNA from a variety of sources in less than an hour
- Minimal genomic DNA contamination of the purified RNA sample
- Reliable performance of the high-quality purified total RNA in downstream applications (see below)
- Compatible with most automated liquid handling workstations

## Downstream Applications

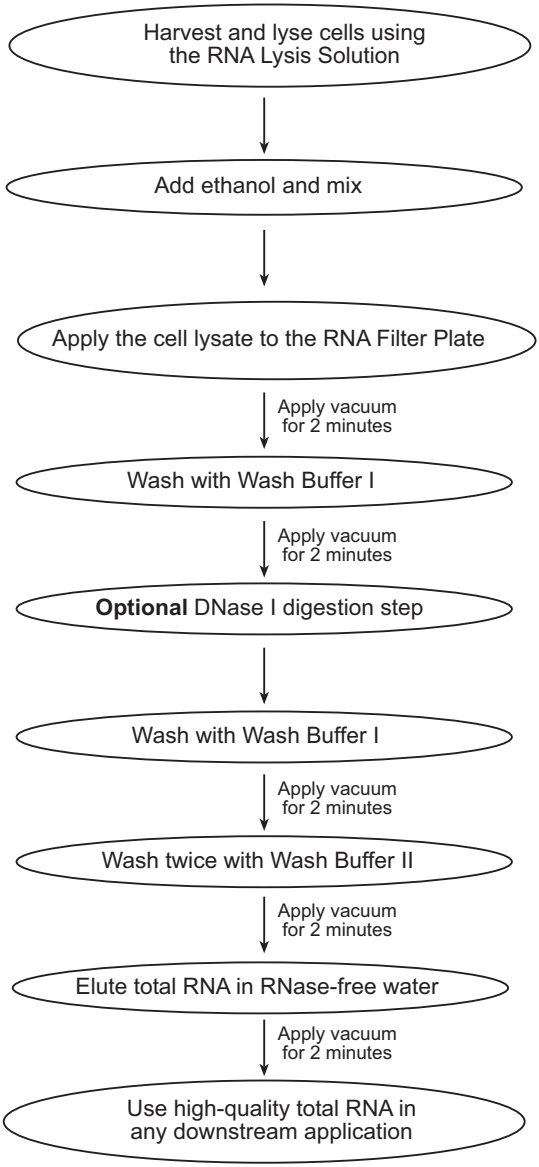
Total RNA isolated using the PureLink™ 96 RNA Purification Kit is suitable for use in applications where the isolated RNA is:

| **Used directly** | **Used after reverse transcription** |
|---|---|
| Northern blotting | RT-PCR |
| Nuclease protection assays | Real time quantitative PCR (qPCR) |
| Reverse transcription | |

# Experimental Overview

## Introduction

The flow chart describes the steps for isolating total RNA.



# Methods

## Preparing Lysates

### Introduction

Instructions for preparing lysates from bacteria, yeast, plant, and mammalian cells and tissue are described below.

To obtain high-quality total RNA, follow the guidelines recommended below for handling RNA.

### Materials Needed

- RNA Lysis Solution (supplied in the kit)
- 70% or 100% ethanol (see appropriate lysis protocol)
- TE Buffer (10 mM Tris-HCl, pH 8, 0.1 mM EDTA)
- Lysozyme and 5% SDS solution (for bacterial lysate)
- Zymolase/lyticase (for yeast lysate)
- Homogenizer/tissue grinder (for plant and tissue lysate)
- Optional β-mercaptoethanol (stock solution, 14.3 M)


The RNA Lysis Solution and Wash Buffer I contains guanidine isothiocyanate. Always wear a laboratory coat, disposable gloves, and eye protection when handling solutions containing this chemical.

Do not add bleach or acidic solutions directly to solutions containing guanidine isothiocyanate or sample preparation waste. Guanidine isothiocyanate forms reactive compounds and toxic gases when mixed with bleach or acids.

### General Handling of RNA

Observe the following guidelines to prevent RNase contamination:

- Use disposable, individually wrapped, sterile plasticware
- Use only sterile, new pipette tips and microcentrifuge tubes
- Wear latex gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin
- Always use proper microbiological aseptic techniques when working with RNA
- Use RNase *AWAY*® Reagent (see page vi) to remove RNase contamination from surfaces

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# Preparing Lysates, Continued

## Optional RNA Lysis Solution

Addition of β-mercaptoethanol (βME) improves cell lysis in some cases. Add 10 µl 14.3 M β-mercaptoethanol to 1 ml of RNA Lysis Solution supplied in the kit and use this solution for cell lysis. Use this solution only where indicated.

## Preparing Bacterial Lysates

Procedure to prepare *E. coli* cell lysate is described below.

1. Harvest up to 1 x $10^9$ *E. coli* cells by centrifugation.
2. Resuspend the pellet in 43 µl TE Buffer. Add lysozyme to the above solution to a final concentration of 1% (w/v). **Note:** You can also prepare 1% lysozyme in TE Buffer and filter-sterilize the solution. Resuspend the pellet in 43 µl of TE Buffer containing 1% lysozyme.
3. Add 1 µl 5% SDS to the lysate, mix, and incubate at room temperature for 5 minutes.
4. Add 150 µl RNA Lysis Solution supplied in the kit (or RNA Lysis Solution with βME) to the lysate followed by the addition of 106 µl 100% ethanol. Mix well.
5. Proceed to isolating RNA (see page 7).

## Preparing Yeast Lysates

Procedure to prepare yeast cell lysate is described below.

1. Harvest 1.8 ml fresh, log-phase yeast cells ($OD_{660}$ = 0.6-0.8) by centrifugation.
2. Resuspend the pellet in 43 µl cold TE Buffer.
3. Add 30 units of zymolase (lyticase) to the above solution and incubate for 30 minutes at 30°C.
4. Add 150 µl RNA Lysis Solution supplied in the kit (or RNA Lysis Solution with βME) to the lysate followed by the addition of 107 µl 100% ethanol. Mix well.
5. Proceed to isolating RNA (see page 7).

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# Preparing Lysates, Continued

## Preparing Plant Lysates

Procedure to prepare lysate from up to 150 mg plant tissue is described below.

1. For hard plant tissue, freeze the tissue in liquid nitrogen and grind the tissue to a powder.

   For soft, non-fibrous plant tissue, cut the tissue into small pieces.
2. Add 150 µl RNA Lysis Solution supplied in the kit (or RNA Lysis Solution with βME) to the tissue from Step 1.
3. Prepare lysate by homogenizing the soft tissue with a homogenizer/tissue grinder or vortexing the ground tissue sample for 1 minute.
4. Centrifuge the lysate at high speed to remove insoluble materials.
5. To 150 µl of lysate, add 150 µl 70% ethanol. Mix well.
6. Proceed to isolating RNA (see page 7).

## Preparing Mammalian Cell Lysates

Cell Growth: Use regular 96-well culture plates (~300 µl capacity) for cell numbers up to 5 x $10^5$ cells. For cell numbers greater than 5 x $10^5$, use 96 deep-well plates (~1 ml capacity).

1. For adherent cells, remove the growth medium from the culture wells.

   For suspension cells, centrifuge the culture plate at 250 x g for 5 minutes to pellet cells. Remove the growth medium.
2. For 5 x $10^5$ cells or less, add 150 µl of RNA Lysis Solution supplied in the kit (or RNA Lysis Solution with βME).

   For > 5 x $10^5$ cells add 350 µl RNA Lysis Solution (or RNA Lysis Solution with βME).
3. Add 150 µl (350 µl for >5 x $10^5$ cells) of 70% ethanol to the cell lysate.
4. Mix the cell lysate by pipetting up and down.
5. Proceed to isolating RNA (see page 7).

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# Preparing Lysates, Continued

### Preparing Tissue Lysates

1. Place ~100 mg of minced mammalian tissue into a 15 ml disposable tube.
2. Add 4.8 ml RNA Lysis Solution to the tube and homogenize the tissue using a homogenizer.
3. Centrifuge the lysate at 2600 x g for 10 minutes at room temperature.
4. Transfer the supernatant to a fresh tube, add 4.8 ml 70% ethanol, and mix.
5. Proceed to isolating RNA (see page 7). You will use 500 µl-900 µl (5-9 mg)* of the tissue lysate per well of the RNA Filter plate.

*The amount of tissue lysate to load into each well of the RNA plate will depend on the tissue type. Viscous tissue lysates or lysates containing high lipid content may clog the filter of the RNA plate. For example, you can load 9 mg of mouse brain lysate into each well of the RNA Filter plate but loading 9 mg of mouse liver lysate will clog the filter.

### Preparing Cells to Isolate Viral RNA

Viral RNA will co-purify with cellular RNA and can be detected and quantitated with qPCR using suitable primers. Use the mammalian cell lysate protocol to prepare a viral cell lysate and perform the RNA isolation as described on page 7.

### Preparing Samples after TRIzol® Extraction

If you isolated total RNA using TRIzol® Reagent and would like to use the RNA samples with the PureLink™ 96 RNA Purification Kit, follow the instructions below:

1. To the upper colorless aqueous phase (containing RNA, obtained after performing phase separation during TRIzol® extraction), add an equal volume of RNA Lysis Buffer and an equal volume of 100% ethanol. Mix well.
2. Proceed to **Binding RNA**, page 8.

**Note**

After preparing the lysate, we recommend isolating RNA immediately. We do not recommend storing the lysate in RNA Lysis Solution at -80°C, as it will adversely affect the quality of your RNA.

# Isolating Total RNA

**Introduction**

Instructions are provided below to isolate total RNA using a vacuum manifold.

**Alternate Protocol**

If you do not have a vacuum manifold, you may use a centrifuge to isolate total RNA using the PureLink™ 96 RNA Purification Kit (see page 16 for detailed protocol).

**Note**

The filter plate format of the PureLink™ 96 RNA Purification Kit is compatible with most automated liquid handling workstations. Small variations in the vacuum manifold design of workstations may require the use an Adapter with the PureLink™ 96 Filter Plates. Contact Technical Service (see page 18) for more information on the Adapter.

**Materials Needed**

- Wash Buffer I and II (supplied in the kit)
- RNase-free water (supplied in the kit)
- 95-100% Ethanol
- Multichannel pipettes and tips
- Vacuum manifold and vacuum pump (producing pressure of 13-15 InHg or -800 to –900 mbar) or automated liquid handling workstations
- DNase I solution (optional, see page 15)

**Before Starting**

Dilute Wash Buffer II (5X) supplied in the kit to 1X with 95-100% ethanol. You will need 200 ml of 1X Wash Buffer II for one 96-well plate.

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# Isolating Total RNA, Continued

## Binding RNA

1. Set up the vacuum manifold using the manufacturer's recommendations.

   If you are using an automated liquid handling workstation, prepare the workstation deck as recommended by the manufacturer. Appropriate methods (scripts) are available for downloading from our Web site at www.invitrogen.com for use with various automated liquid handling workstations.
2. Place the RNA Filter Plate on the vacuum manifold. Transfer lysates (prepared as described on pages 3-6) to the RNA Filter Plate.
3. Apply vacuum for 2 minutes at room temperature. Release vacuum.
4. Proceed to **Washing RNA**, next page.

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# Isolating Total RNA, Continued

## Washing RNA

1. Add 500 µl of Wash Buffer I (supplied in the kit) to each well of the RNA Filter Plate. Apply vacuum for 2 minutes at room temperature. Release vacuum. Proceed to DNase digestion if you need to remove genomic DNA or proceed directly to Step 2.

   **DNase Digestion (Optional):** To remove genomic DNA from the samples, add 80 µl of DNase I solution (see page 15 for a recipe) into each well of the RNA Filter Plate and apply vacuum briefly to allow the solution to soak into the filter matrix. Incubate the plate at room temperature for 15 minutes.
2. Add 500 µl of Wash Buffer I to each well of the RNA Filter Plate. If DNase I digestion is performed, incubate for 5 minutes at room temperature.
3. Apply vacuum for 2 minutes at room temperature. Release vacuum.
4. Add 1 ml of **1X Wash Buffer II** to the RNA Filter Plate and apply vacuum for 2 minutes at room temperature. Release vacuum. Repeat this wash step one more time.
5. After releasing vacuum, place the Filter Plate with the filter side down on a stack of paper towels and firmly pat dry the plate.
6. Place the RNA Filter Plate on the vacuum manifold and apply vacuum for 5-10 minutes at room temperature. Release vacuum.
7. Proceed to **Eluting RNA**, below.

## Eluting RNA

1. Place the Receiver Plate included in the kit in the vacuum manifold (in place of the waste collection tray) and place the RNA Filter Plate on top of the Receiver Plate.
2. Add 170 µl of RNase-free Water supplied in the kit to each well of the RNA Filter Plate. Incubate for 1 minute at room temperature.
3. Apply vacuum for 2 minutes at room temperature. Release vacuum. The RNA is eluted into the Receiver Plate. The elution volume is 140-150 µl.
4. Store RNA in the Receiver Plate or transfer RNA to RNase-free tubes and store at -80°C.

Determine the quality and quantity of the purified RNA as described on the next page.

# Determining the RNA Quality and Quantity

## Introduction

Once you have isolated total RNA, determine the quantity and quality of the purified RNA as described in this section.

## Estimating RNA Quantity

Use a spectrophotometer to determine the quantity of the purified total RNA by UV absorbance at $OD_{260}$.

1. Dilute an aliquot of the total RNA sample in 10 mM Tris-HCl, pH 7.0. Mix well. Transfer to a cuvette (1-cm path length).

   **Note:** The RNA must be in a neutral pH buffer to accurately measure the UV absorbance.

2. Determine the $OD_{260}$ of the solution using a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.0.
3. Calculate the amount of total RNA using the following formula:

$$\text{Total RNA } (\mu g) = OD_{260} \times [40\ \mu g/(1\ OD_{260} \times 1\ ml)] \times \text{dilution factor} \times \text{total sample volume (ml)}$$

**Example:**

Total RNA was eluted in water in a total volume of 150 µl. A 40 µl aliquot of total RNA was diluted to 500 µl in 10 mM Tris-HCl, pH 7.5. An $OD_{260}$ of 0.188 was obtained. The amount of RNA in the sample is determined as shown below:

$$\text{Total RNA } (\mu g) = 0.188 \times [40\ \mu g/(1\ OD_{260} \times 1\ ml)] \times 12.5 \times 0.15$$

$$= 14.1\ \mu g \text{ of total RNA}$$

*Continued on next page*

# Determining the RNA Quality and Quantity, Continued

## Analyzing RNA Quality

Typically, RNA isolated using the PureLink™ 96 RNA Purification Kit has an $OD_{260/280}$ of >1.8 when samples are diluted in Tris-HCl (pH 7.5). An $OD_{260/280}$ of >1.8 indicates that RNA is reasonably clean of proteins and other UV chromophores (heme, chlorophyl, etc.) that could either interfere with downstream applications or negatively affect the stability of the stored RNA.

Agarose gel electrophoresis of RNA isolated using the PureLink™ 96 RNA Purification Kit shows the 28S to 18S band ratio to be >1.5. RNA is judged to be intact if discreet 28S and 18S ribosomal RNA bands are observed.

Gel analysis reveals if contaminating DNA is present either as a band at the well or between the well and 28S band or as some background smearing. Contaminating DNA is easily removed by treating the RNA samples with DNase I during purification or after eluting the RNA.

The ribosomal RNA sizes from various sources are listed below:

| Source | 16S/18S | 23S/28S |
|---|---|---|
| *E. coli* | 1.5 kb | 2.9 kb |
| *S. cerevisiae* | 1.8 kb | 3.4 kb |
| Mouse | 1.9 kb | 4.7 kb |
| Human | 1.9 kb | 5.0 kb |

## The Next Step

Total RNA isolated using the PureLink™ 96 RNA Purification Kit is suitable for use in any downstream application of choice (RT-PCR, reverse transcription, and qPCR reactions) using kits available from Invitrogen (see page vi) without the need to perform any additional steps.

# Expected Results

## Introduction

The quantity and quality of total RNA obtained using the PureLink™ 96 RNA Purification Kit are described below.

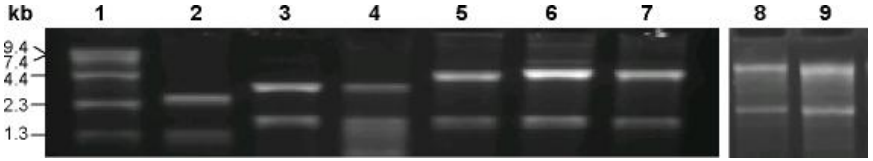
## RNA Yield

The yield of total RNA obtained from various sources is listed below:

| Material | Amount | Total RNA Yield |
|---|---|---|
| Bacteria (*E. coli*) | 5 x $10^8$ cells | 3.74 µg |
| Yeast (*S. cerevisiae*) | 2 x $10^8$ cells | 33.59 µg |
| Plant (Lettuce) | 100 mg | 10.33 µg |
| Mammalian | | |
| Human HeLa cells | 3.7 x $10^5$ | 9.56 µg |
| Human 293 cells | 3.7 x $10^5$ | 16.91 µg |
| Mouse NIH3T3 cells | 3.7 x $10^5$ | 14.38 µg |
| Mouse Liver | 5 mg | 18.2 µg |
| Mouse Brain | 9 mg | 5.4 µg |

## RNA Integrity

Total RNA isolated from various sources was analyzed by agarose gel electrophoresis and stained with ethidium bromide. The gel shows 23S/28S and 16S/18S bands in a ratio >1.5 with minimal DNA contamination.




Lane 1: 0.24 – 9.5 Kb RNA Ladder
Lane 2: 0.7 µg of total RNA from 5 x $10^8$ *E.coli* cells
Lane 3: 0.8 µg of total RNA from 2 x $10^8$ *S. cerevisiae* cells
Lane 4: 1.0 µg of total RNA from 100 mg lettuce
Lane 5: 1.1 µg of total RNA from mouse 3.7 x $10^5$ NIH3T3 cells
Lane 6: 1.4 µg of total RNA from 3.7 x $10^5$ human 293 cells
Lane 7: 1.0 µg of total RNA from 3.7 x $10^5$ human HeLa cells
Lane 8: 0.5 µg of total RNA from 9 mg mouse brain
Lane 9: 0.5 µg of total RNA from 5 mg mouse liver

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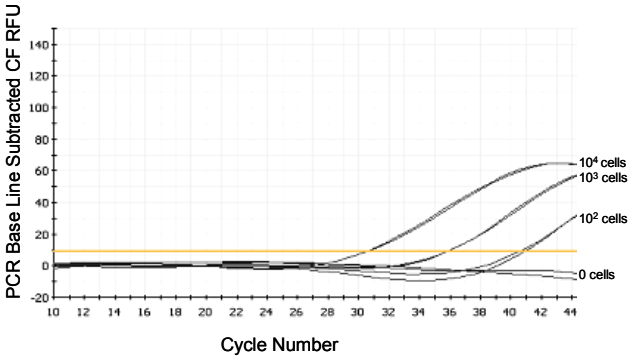
# Expected Results, Continued

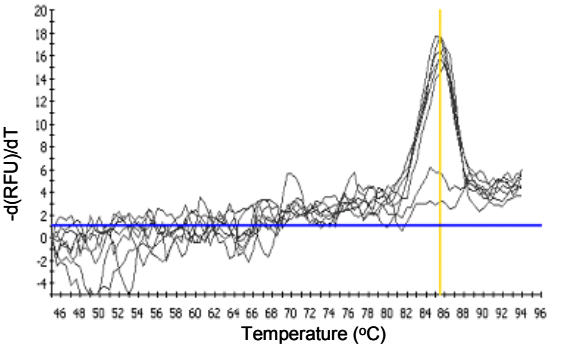
## Quantitative PCR (qPCR) Results

The human SDHA (Succinate Dehydrogenase, subunit A) transcript was quantified from total RNA isolated from human HeLa cells (0, 100, 1000, and 10,000 cells). 5 µl of total RNA from each sample was used for analysis in duplicate. The one-step real time RT-PCR reaction was performed with FAM-labeled LUX™ primer (forward) and unlabeled primer (reverse) using the Platinum® Quantitative RT-PCR Thermoscript™ One-Step System (see page vi).

**Gene-specific Real Time RT-PCR of RNA**




**Melting Temperature Analysis of Amplified hSDHA PCR Product**



# Troubleshooting

## Introduction

Review the information below to troubleshoot your experiments with the PureLink™ 96 RNA Purification Kit.

To troubleshoot problems with the vacuum manifold or automated liquid handling workstations, contact the manufacturer.

| Problem | Cause | Solution |
|---|---|---|
| Low RNA yield | Incomplete lysis or too much cell lysate has clogged the filter | Decrease the lysate volume used. Increase the volume of RNA Lysis Solution for mammalian cells >5 x $10^5$ to achieve complete lysis. Using RNA Lysis Solution with β-mercaptoethanol improves cell lysis. |
| RNA degraded | RNA contaminated with RNase | Follow the guidelines on page 3 to prevent RNase contamination. |
| Genomic DNA contamination | -- | Use optional DNase I digestion step included in the protocol to remove genomic DNA contamination.<br>You can also perform DNase I digestion after the RNA elution step. |
| Low elution volume or sample cross-contamination | Incorrect vacuum pressure | Make sure the vacuum manifold is sealed tightly and there is no leakage. A vacuum pressure of –800 to –900 mbar (13-15 InHg) is required to obtain the best results. |

# Appendix

# Recipes

### DNase I Solution

DNase I is available from Invitrogen (catalog no. 18047-019). DNase I, Amplification Grade (catalog no. 18068-015) is also available from Invitrogen (see **Note** below).

You will need 8 ml of DNase I solution for each 96 well plate. Use freshly prepared DNase I solution.

Prepare 8 ml of DNase I solution as follows:

1. Prepare 10X DNase I Buffer using RNase-free water to yield the following final concentration:

   200 mM Tris-HCl, pH 8.4

   20 mM $MgCl_2$

   500 mM KCl

2. To a sterile RNase-free tube, add:

| | |
|---|---|
| 10X DNase I Buffer (from Step 1) | 0.8 ml |
| DNase I (catalog no. 18047-019) | 3200 units |
| RNase-free water | to 8 ml |

3. Mix the contents and use this solution for DNase I digestion.

**Note:** You will use 32 units of DNase I per well for DNase digestion. High concentration of DNase I is used during the DNase I digestion step since the enzyme may not be fully active in the presence of salts and chelating agents from the RNA Lysis Solution. The high concentration of DNase I will not cause any RNA degradation.

**Note**

DNase I, Amplification Grade (catalog no. 18068-015) is available from Invitrogen (see page vi) and is supplied with a vial of 10X DNase I reaction buffer (200 mM Tris-HCl, pH 8.4, 20 mM $MgCl_2$, 500 mM KCl). If you are using this enzyme, there is no need to prepare the 10X DNase I buffer as described above.

# RNA Isolation Using Centrifugation

**Introduction**

An alternate protocol is provided below to isolate total RNA using centrifugation, if you do not have a vacuum manifold.

**Materials Needed**

- Wash Buffer I and II (supplied in the kit)
- RNase-free water (supplied in the kit)
- 95-100% Ethanol
- Multichannel pipettes and tips
- PureLink™ 96 Receiver Plates (see **Note** below)
- Centrifuge capable of centrifuging 96-well plates (stacked plates have a plate height of 6.5 cm).
- DNase I solution (optional, see page vi)

**Note**

The Receiver Plates supplied with the PureLink™ 96 RNA Purification Kit are not compatible for use with centrifugation. You will need deep-well PureLink™ 96 Receiver Plates (see page vi for ordering information).

**Before Starting**

Dilute Wash Buffer II (5X) supplied in the kit to 1X with 95-100% ethanol. You will need 150 ml of 1X Wash Buffer II for one 96-well plate.

**Binding RNA**

1. Prepare lysates as described on page 3.
2. Place the RNA Filter Plate on top of a Receiver Plate. Transfer cell lysates to the RNA Plate.
3. Centrifuge the stacked plates at 3000 x g for 1-2 minutes at room temperature.
4. Discard flow-through from the Receiver Plate and place the RNA Filter Plate on top of the Receiver Plate. Proceed to **Washing RNA**, next page.

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# RNA Isolation Using Centrifugation,
Continued

## Washing RNA

1. Add 500 µl of Wash Buffer I supplied in the kit to each well of the RNA Filter Plate.
2. Centrifuge the stacked plates at 3000 x g for 1-2 min at room temperature. Discard flow-through and place the RNA Filter Plate on top of the Receiver Plate. Proceed to DNase digestion if you need to remove genomic DNA or proceed directly to Step 3.

   **DNase Digestion (Optional):** To remove genomic DNA from the samples, add 80 µl of DNase I solution (see page 15 for a recipe) into each well of the RNA Filter Plate and incubate at room temperature for 15 minutes.
3. Add 500 µl of Wash Buffer I supplied in the kit to each well of the RNA Filter Plate. If DNase I digestion is performed, incubate for 5 minutes at room temperature.
4. Centrifuge the stacked plates at 3000 x g for 1-2 min at room temperature. Discard flow-through.
5. Add 750 µl of **1X Wash Buffer II** to each well of the RNA Filter Plate.
6. Centrifuge the stacked plates at 3000 x g for 1-2 minutes at room temperature. Discard flow-through and repeat Steps 5-6 once.
7. Centrifuge the RNA Filter plate at 3000 x g for 5 minutes to dry after the second 1X Wash Buffer II wash.
8. Proceed to **RNA Elution**, below.

## RNA Elution

1. Place the RNA Filter Plate on top of a **new** Receiver Plate.
2. Add 45 µl of RNase-free Water supplied in the kit to each well of the RNA Filter Plate. Incubate for 1 minute at room temperature.
3. Centrifuge the stacked plates at 3000 x g for 1-2 minutes at room temperature to elute RNA.
4. Store RNA in the collection plate or transfer RNA to RNase-free tubes and store at -80°C.

Determine the quality and quantity of the purified RNA as described on page 10. Total RNA isolated using the PureLink™ 96 RNA Purification System is suitable for use in any downstream application of choice (see page 1).

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# Technical Service

## World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

**http://www.invitrogen.com**

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

## Contact Us

For more information or technical assistance, please call, write, fax, or email. Additional international offices are listed on our web page (www.invitrogen.com).


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## MSDS Requests

To request an MSDS, visit our Web site at www.invitrogen.com. On the home page, go to 'Technical Resources' and select 'Search Technical Resources'. Use the product name or catalog no. and search all technical resources or check the box and search just MSDSs.

# Purchaser Notification

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