USER GUIDE

ambion®
by life technologies™

PureLink® Pro 96 RNA Purification Kit

For high-throughput purification of total RNA

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For Research Use Only. Not for use in diagnostic procedures.

life technologies™

Contents

Kit Contents and Storage

Shipping and Storage

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

Contents

The components included in the PureLink® Pro 96 RNA Purification Kit are listed in the following table. Sufficient reagents are included in the kit to perform 384 (4 × 96) isolations.

Item	Amount
PureLink® Pro 96 Lysis Buffer	150 mL
PureLink® Pro 96 Wash Buffer I	450 mL
PureLink® Pro 96 Wash Buffer II (5X)	175 mL
PureLink® Pro 96 RNase-free Water	75 mL
PureLink® 96 Well Total RNA Filter Plates	4 Plates
PureLink® Pro 96 Elution Plates	4 Plates

Introduction

About the Kit

Introduction

The PureLink® Pro 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® Pro 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 PureLink® Pro 96 Lysis Buffer 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® Pro 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
- Isolation of total RNA from a variety of sources in less than an hour
- Minimal genomic DNA contamination
- Reliable performance of purified RNA in downstream applications
- Compatible with most automated liquid handling workstations

Downstream Applications

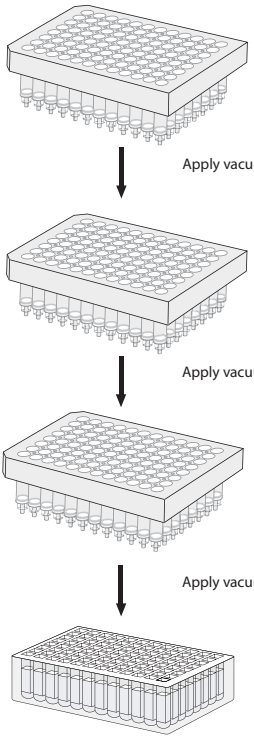
Total RNA isolated using the PureLink® Pro 96 RNA Purification Kit is suitable for use in applications where the isolated RNA is:

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

Experimental Overview

Workflow

The procedure for isolating total RNA after lysis of samples with PureLink® Pro 96 Lysis Buffer is shown in the following flow chart.



Methods

Preparing Lysates

Introduction

Instructions for preparing lysates from bacteria, yeast, plant, and mammalian cells and tissue are provided in the following sections.

Materials Needed

- PureLink® Pro 96 Lysis Buffer (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 PureLink® Pro 96 Lysis Buffer 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
- Always use proper microbiological aseptic techniques when working with RNA
- Use RNase *AWAY*® Reagent (see page 26) to remove RNase contamination from surfaces (including from non-disposable equipment like homogenizers)

Note

Isolate RNA immediately after preparing the lysate. Do not store the lysate in PureLink® Pro 96 Lysis Buffer, as it will adversely affect the quality of your RNA.

Preparing Lysates, Continued

Optional RNA Lysis Solution

Addition of β-mercaptoethanol (β-ME) improves cell lysis in some cases. Add 10 µL 14.3 M β-mercaptoethanol to 1 mL of PureLink® Pro 96 Lysis Buffer and use this solution for cell lysis as directed.

Preparing Bacterial Lysates

Preparation of *E. coli* cell lysates:

1. Harvest up to 1×10^9 *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 PureLink® Pro 96 Lysis Buffer (or PureLink® Pro 96 Lysis Buffer with β-ME) to the lysate followed by 106 µL of 100% ethanol. Mix well.
5. Proceed to isolating RNA as described on page 11 (Centrifugation), page 13 (Vacuum Manifold), or page 23 (EveryPrep™ Vacuum Manifold).

Preparing Yeast Lysates

Preparation of yeast cell lysates:

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 of cold TE Buffer.
3. Add 30 units of zymolase (lyticase) and incubate for 30 minutes at 30°C.
4. Add 150 µL PureLink® Pro 96 Lysis Buffer (or PureLink® Pro 96 Lysis Buffer with β-ME) to the lysate followed by 107 µL of 100% ethanol. Mix well.
5. Proceed to isolating RNA as described on page 11 (Centrifugation), page 13 (Vacuum Manifold), or page 23 (EveryPrep™ Vacuum Manifold).

Preparing Lysates, Continued

Preparing Plant Lysates

Preparation of lysate from up to 150 mg plant tissue:

1. Freeze hard plant tissue in liquid nitrogen and grind to a powder.

   Cut soft, non-fibrous plant tissue into small pieces.
2. Add 150 µL PureLink® Pro 96 Lysis Buffer (or PureLink® Pro 96 Lysis Buffer with β-ME) to the tissue.
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. Add 150 µL 70% ethanol to 150 µL of lysate. Mix well.
6. Proceed to isolating RNA as described on page 11 (Centrifugation), page 13 (Vacuum Manifold), or page 23 (EveryPrep™ Vacuum Manifold).

Preparing Mammalian Cell Lysates

Preparation of lysate from mammalian cells:

1. Grow up to 5×10^5 cells in regular 96-well culture plates (~300 µL capacity).
   For cell numbers greater than 5×10^5, use 96 deep-well plates (~1 mL capacity).
2. For adherent cells, remove the growth medium from the culture wells.

   For suspension cells, centrifuge the culture plate at $250 \times g$ for 5 minutes to pellet cells. Remove the growth medium.
3. For $<5 \times 10^5$ cells, add 150 µL of PureLink® Pro 96 Lysis Buffer (or PureLink® Pro 96 Lysis Buffer with β-ME).

   For $>5 \times 10^5$ cells add 350 µL PureLink® Pro 96 Lysis Buffer (or PureLink® Pro 96 Lysis Buffer with β-ME).
4. Add 150 µL (350 µL for $>5 \times 10^5$ cells) of 70% ethanol to the cell lysate.
5. Mix the cell lysate by pipetting up and down.
6. Proceed to isolating RNA as described on page 11 (Centrifugation), page 13 (Vacuum Manifold), or page 23 (EveryPrep™ Vacuum Manifold).

Preparing Lysates, Continued

Preparing Tissue Lysates

Preparation of lysate from 100 mg tissue:

1. Place ~100 mg of minced mammalian tissue into a 15-mL disposable tube.
2. Add 4.8 mL PureLink® Pro 96 Lysis Buffer to the tube and homogenize the tissue.
3. Centrifuge the lysate at 2600 × *g* for 10 minutes at room temperature.
4. Transfer the supernatant to a fresh tube, add 4.8 mL 70% ethanol, and mix.

   Proceed to isolating RNA as described on page 11 (Centrifugation), page 13 (Vacuum Manifold), or page 23 (EveryPrep™ Vacuum Manifold). Use 500 µL–900 µL (5–9 mg)* of the tissue lysate per well of the PureLink® 96 Well Total RNA Filter Plate.

*The amount of lysate depends on the tissue type. Viscous lysates or lysates containing high lipid content may clog the PureLink® RNA Filter Plate.

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 11 (Centrifugation), page 13 (Vacuum Manifold), or page 23 (EveryPrep™ Vacuum Manifold).

Preparing Samples after TRIzol® Extraction

When using total RNA isolated with TRIzol® Reagent use the following protocol:

1. Collect the upper colorless aqueous phase (containing RNA) after performing phase separation during the TRIzol® extraction.
2. Add a volume of PureLink® Pro 96 Lysis Buffer equal to the aqueous phase.
3. Add a volume of 100% ethanol equal to the aqueous phase.
4. Mix well.
5. Proceed to **Binding RNA** as described on page 11 (Centrifugation), page 13 (Vacuum Manifold), or page 23 (EveryPrep™ Vacuum Manifold).

RNA Isolation Using Centrifugation

Introduction

A protocol is provided below to isolate total RNA using centrifugation. Perform all steps at room temperature.

Materials Needed

- PureLink® Pro 96 Wash Buffer I and II (supplied in kit)
- RNase-free water (supplied in kit)
- 95–100% Ethanol
- Multichannel pipettes and tips
- PureLink® Pro 96 Elution Plates
- PureLink® 96 Receiver Plates (see page 26)
- Centrifuge with a swinging bucket rotor with plate carriers that have a plate height clearance of 7.0 cm
- DNase I solution (optional, see page 20)

Before Starting

Dilute PureLink® Pro 96 Wash Buffer II (5X) to 1X with 95–100% ethanol. Use 150 mL of 1X PureLink® Pro 96 Wash Buffer II for one 96-well plate.

Binding RNA

1. Prepare lysates as described on page 7–10.
2. Place the PureLink® 96 Well Total RNA Filter Plate on top of a PureLink® 96 Receiver Plate. Transfer cell lysates to the PureLink® RNA Filter Plate.
3. Centrifuge the stacked plates at ≥2,100 × *g* for 1–2 minutes at room temperature.
4. Discard flow-through from the PureLink® Receiver Plate and place the PureLink® RNA Filter Plate on top of the PureLink® Receiver Plate. Proceed to **Washing RNA**, page 12.

RNA Isolation Using Centrifugation,
Continued

Washing RNA

1. Add 500 µL of PureLink® Pro 96 Wash Buffer I to each well of the PureLink® RNA Filter Plate.
2. Centrifuge the stacked plates at ≥2100 × *g* for 1–2 minutes. Discard flow-through and place the PureLink® RNA Filter Plate back on top of the PureLink® Receiver Plate.

   Note: An optional on-column DNase digestion can be performed at this point to remove genomic DNA see page 20.
3. Add 750 µL of **1X** PureLink® Pro 96 Wash Buffer II to each well of the PureLink® RNA Filter Plate.
4. Centrifuge the stacked plates at ≥2100 × *g* for 1–2 minutes. Discard flow-through and replace the PureLink® RNA Filter Plate.
5. Add 750 µL of **1X** PureLink® Pro 96 Wash Buffer II to each well of the PureLink® RNA Filter Plate.
6. Centrifuge the stacked plates at ≥2100 × *g* for 1–2 minutes. Discard flow-through and replace the PureLink® RNA Filter Plate.
7. Dry the PureLink® RNA Filter Plate by centrifugation at ≥2100 × *g* for 10 minutes.
8. Proceed to **RNA Elution**.

RNA Elution

1. Place the RNA Filter Plate on top of a **new** PureLink® Pro 96 Elution Plate.
2. Add 45 µL of RNase-free Water (supplied in the kit) to each well of the PureLink® RNA Filter Plate. Incubate for 1 minute at room temperature.
3. Centrifuge the stacked plates at ≥2100 × *g* for 1–2 minutes to elute RNA.
4. Store RNA in the PureLink® Elution 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 15.

RNA Isolation Using a Vacuum Manifold

Introduction

Instructions are provided below to isolate total RNA using a vacuum manifold. All steps are performed at room temperature. For a protocol using the EveryPrep™ Universal Vacuum Manifold, see page 22.

Materials Needed

- PureLink® Pro 96 Wash Buffer I and II (supplied in kit)
- RNase-free water (supplied in kit)
- 95–100% Ethanol
- Multichannel pipettes and tips
- Vacuum manifold and vacuum pump (producing pressure of 12–15 in. Hg) or automated liquid handling workstations
- DNase I solution (optional, see page 20)

Before Starting

Dilute PureLink® Pro 96 Wash Buffer II (5X) to 1X with 95–100% ethanol. Use 200 mL of 1X PureLink® Pro 96 Wash Buffer II for one 96-well plate.

Binding RNA

1. Set up the vacuum manifold according to 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 website at **www.lifetechnologies.com** for use with various automated liquid handling workstations.
2. Place the PureLink® RNA Filter Plate on the vacuum manifold. Transfer lysates (prepared as described on pages 7–10) to the RNA Filter Plate.
3. Apply vacuum for 2 minutes at room temperature. Release vacuum.
4. Proceed to **Washing RNA**, page 14.

RNA Isolation Using a Vacuum Manifold,
Continued

Washing RNA

1. Add 500 µL of PureLink® Pro 96 Wash Buffer I (supplied in the kit) to each well of the PureLink® RNA Filter Plate.
2. Apply vacuum for 2 minutes. Release vacuum.

   Note: An optional on-column DNase digestion can be performed at this point to remove genomic DNA see page 20.
3. Add 1 mL of **1X** PureLink® Pro 96 Wash Buffer II to the PureLink® RNA Filter Plate and apply vacuum for 2 minutes. Release vacuum.
4. Add 1 mL of **1X** PureLink® Pro 96 Wash Buffer II to the PureLink® RNA Filter Plate and apply vacuum for 2 minutes. Release vacuum.
5. Place the PureLink® RNA Filter Plate with the filter side down on a stack of paper towels, and pat firmly to blot residual liquid.
6. Place the PureLink® RNA Filter Plate on the vacuum manifold and apply vacuum for 5–10 minutes. Release vacuum.
7. Proceed to **Eluting RNA**.

Eluting RNA

1. Place the PureLink® Pro 96 Elution Plate in the vacuum manifold (in place of the waste collection tray) and place the RNA Filter Plate on top of the PureLink® Elution Plate.
2. Add 170 µL of RNase-free Water (supplied in the kit) to each well of the PureLink® RNA Filter Plate. Incubate for 1 minute at room temperature.
3. Apply vacuum for 2 minutes. Release vacuum. The RNA is eluted into the PureLink® Elution Plate. The elution volume is 140–150 µL.
4. Store RNA in the PureLink® Elution 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 15.

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 calculated as shown:

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

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

Determining the RNA Quality and Quantity, Continued

Analyzing RNA Quality

Typically, RNA isolated using the PureLink® Pro 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® Pro 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 the presence of contaminating DNA 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® Pro 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 Life Technologies (see page 26) without the need to perform any additional steps.

Expected Results

Introduction

The quantity and quality of total RNA obtained using the PureLink® Pro 96 RNA Purification Kit are described in the following table.

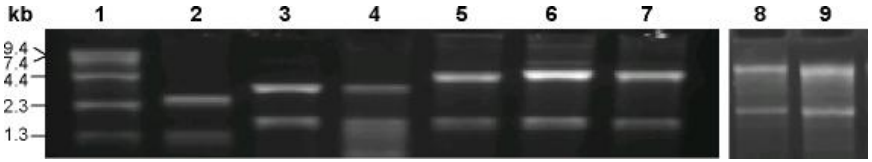
RNA Yield

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

Material	Amount	Total RNA Yield
Bacteria (*E. coli*)	5×10^8 cells	3.74 µg
Yeast (*S. cerevisiae*)	2×10^8 cells	33.59 µg
Plant (Lettuce)	100 mg	10.33 µg
Mammalian		
Human HeLa cells	3.7×10^5	9.56 µg
Human 293 cells	3.7×10^5	16.91 µg
Mouse NIH3T3 cells	3.7×10^5	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.



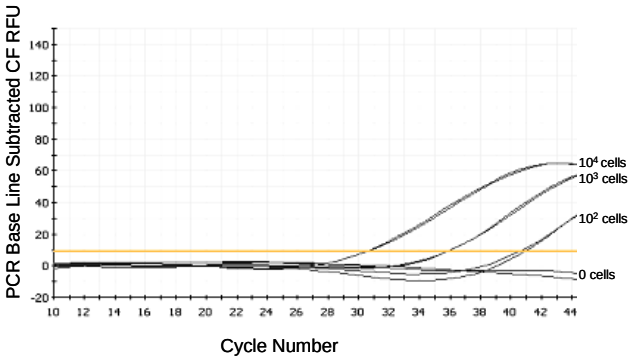
Lane 1: 0.24–9.5 Kb RNA Ladder
Lane 2: 0.7 µg of total RNA from 5×10^8 *E.coli* cells
Lane 3: 0.8 µg of total RNA from 2×10^8 *S. cerevisiae* cells
Lane 4: 1.0 µg of total RNA from 100 mg lettuce
Lane 5: 1.1 µg of total RNA from mouse 3.7×10^5 NIH3T3 cells
Lane 6: 1.4 µg of total RNA from 3.7×10^5 human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

Expected Results, Continued

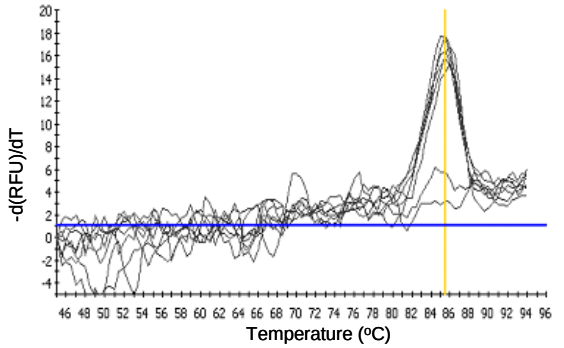
Quantitative PCR (qPCR) Results

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 26).

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® Pro 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 PureLink® Pro 96 Lysis Buffer for mammalian cells >5 × 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 7 to prevent RNase contamination.
Genomic DNA contamination	—	Use the optional DNase I digestion step included in the Appendix to remove genomic DNA contamination. Perform DNase I digestion after the RNA elution step (see Appendix).
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 12–15 in. Hg is required to obtain the best results.

Appendix

On-Column DNase Digestion

Introduction

If it is necessary to remove genomic DNA from samples, an on-column DNase digestion can be performed between wash steps with PureLink® Pro 96 Wash Buffer I and PureLink® Pro 96 Wash Buffer II. All steps are performed at room temperature.

Materials Needed

- DNase I (see page 26)
- RNase-free water

Preparing Solutions

Prepare 8 mL of DNase I solution for each 96 well plate. Always use freshly prepared DNase I solution.

Prepare 8 mL of DNase I solution in a sterile RNase-free tube as follows:

1. 10X DNase I Buffer (see page 21)	0.8 mL
DNase I	3200 units
RNase-free water	to 8 mL

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

Note: Use 32 units of DNase I per well for the on-column DNase digestion protocol. A high concentration of DNase I is used for DNase I digestion since the enzyme may not be fully active in the presence of salts and chelating agents from the PureLink® Pro 96 Lysis Buffer. This concentration of DNase I does not cause RNA degradation.

Note

DNase I, Amplification Grade (Cat. no. 18068-015) from Life Technologies (see page 26) 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 in **Preparing Solutions**.

On-Column DNase Digestion, Continued

10X DNase I Buffer

10X DNase I Buffer

200 mM Tris-HCl, pH 8.4

20 mM $MgCl_2$

500 mM KCl

Prepare 1 mL of 10X DNase I Buffer using RNase-free water as follows:

1. 1 M Tris-HCl, pH 8.4	200 µL
100 mM $MgCl_2$	200 µL
1 M KCl	500 µL
RNase-free water	to 8 mL

DNase I Digestion: Centrifugation Protocol

1. Add 80 µL of DNase I solution (see page 20 for recipe) into each well of the PureLink® RNA Filter Plate and incubate at room temperature for 15 minutes.
2. Add 500 µL of PureLink® Pro 96 Wash Buffer I to each well of the PureLink® RNA Filter Plate and incubate for an additional 5 minutes.
3. Centrifuge the stacked plates at ≥2100 × *g* for 1–2 minutes. Discard flow-through and place the PureLink® RNA Filter Plate back on top of the PureLink® Receiver Plate.
4. Proceed with **Washing RNA** Step 3, page 12.

DNase I Digestion: Vacuum Manifold Protocol

DNase I digestion protocol for vacuum manifolds, including the EveryPrep™ Vacuum Manifold:

1. Add 80 µL of DNase I solution (see page 20 for recipe) into each well of the PureLink® 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 PureLink® Pro 96 Wash Buffer I to each well of the PureLink® RNA Filter Plate and incubate for an additional 5 minutes.
3. Apply vacuum for 2 minutes. Release vacuum.
4. Proceed with **Washing RNA** Step 3; page 14 (Vacuum Manifold), or page 24 (EveryPrep™ Vacuum Manifold).

RNA Isolation Using the EveryPrep™ Universal Vacuum Manifold

Introduction

Use the provided instructions to isolate total RNA using the EveryPrep™ Universal Vacuum Manifold (see page 26). Refer to the manual for the EveryPrep™ Universal Vacuum Manifold for detailed instructions on operation with the 96 Well Top Plate. All steps are performed at room temperature.

Materials Needed

- PureLink® Pro 96 Wash Buffer I and II (supplied in kit)
- RNase-free water (supplied in kit)
- 95–100% Ethanol
- Multichannel pipettes and tips
- Vacuum manifold and vacuum pump (producing pressure of 12–15 in. Hg)
- DNase I solution (optional, see page 20)

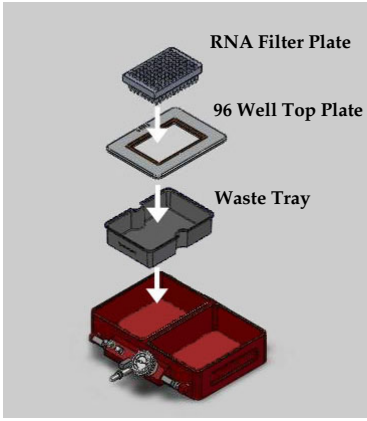
Before Starting

Dilute PureLink® Pro 96 Wash Buffer II (5X) to 1X with 95–100% ethanol. Use 200 mL of 1X PureLink® Pro 96 Wash Buffer II for one 96-well plate.

RNA Isolation Using the EveryPrep™ Universal Vacuum Manifold, Continued

EveryPrep™ Universal Vacuum Manifold Assembly

1. Assemble the EveryPrep™ Universal Vacuum Manifold: Place the Waste Tray in the Binding Chamber, cover the top with the 96 Well Top Plate, and place the PureLink® RNA Filter Plate over the Top Plate.



2. Proceed to **Binding RNA**.

Binding RNA

1. Transfer lysates (prepared as described on pages 7–10) to the PureLink® RNA Filter Plate.
2. Apply vacuum for 2 minutes at room temperature. Release vacuum.
3. Proceed to **Washing RNA**, page 24.

RNA Isolation Using the EveryPrep™ Universal Vacuum Manifold, Continued

Washing RNA

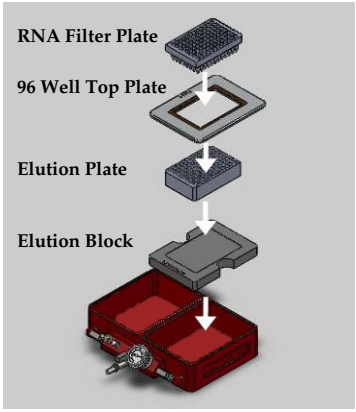
1. Add 500 µL of PureLink® Pro 96 Wash Buffer I to each well of the PureLink® RNA Filter Plate.
2. Apply vacuum for 2 minutes. Release vacuum.

   Note: An optional on-column DNase digestion can be performed at this point to remove genomic DNA see page 20.
3. Add 1 mL of **1X PureLink® Pro 96 Wash Buffer II** to the PureLink® RNA Filter Plate.
4. Apply vacuum for 2 minutes. Release vacuum.
5. Add 1 mL of **1X PureLink® Pro 96 Wash Buffer II** to the PureLink® RNA Filter Plate.
6. Apply vacuum for 2 minutes. Release vacuum.
7. Place the PureLink® RNA Filter Plate with the filter side down on a stack of paper towels, and pat firmly to blot residual liquid.
8. Apply vacuum for 5 minutes. Release vacuum.
9. Proceed to **Eluting RNA**, page 25.

RNA Isolation Using the EveryPrep™ Universal Vacuum Manifold, Continued

Eluting RNA

1. Prepare the EveryPrep™ Universal Vacuum Manifold for elution: Place the Elution Block and PureLink® Pro 96 Elution Plate in the Elution Chamber, cover the top with the 96 Well Top Plate, and place the PureLink® RNA Filter Plate over the Top Plate.



2. Add 170 µL of RNase-free Water (supplied in the kit) to each well of the PureLink® 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 PureLink® Elution Plate. The elution volume is 140–150 µL.
4. Store RNA in the PureLink® Elution 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 15.

Accessory Products

Additional Products

The following products are also available from Life Technologies. For more details on these products, visit **www.lifetechnologies.com** or contact Technical Support (page 27).

Product	Quantity	Catalog No.
PureLink® 96 RNA Components Available Separately		
PureLink® 96 Receiver Plates (deep-well)	50 pack	12193-025
PureLink® 96 RNA Lysis Buffer	750 mL	12173-022
PureLink® 96 RNA Wash Buffer I (1X)	5 × 1 L	12173-032
PureLink® 96 RNA Wash Buffer II (5X)	2 × 1 L	12173-033
PureLink® 96 RNA Filter Plates	50 pack	12173-035
PureLink® 96 RNA Mini Kit	50 preps	12183018A
	10 preps	12183020
Vacuum Manifold		
EveryPrep™ Universal Vacuum Manifold	1 manifold	K2111-01
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
Reagents		
RNase *AWAY*®	250 mL	10328-011
RNase*ZAP*®	250 mL	AM9780
DNase I	20,000 units	18047-019
DNase I, Amplification Grade	100 units	18068-015
0.1–2 Kb RNA Ladder	75 µg	15623-100
0.5–10 Kb RNA Ladder	75 µg	15623-200
UltraPure™ DEPC-treated Water	1 L	750023
UltraPure™ DNase/RNase-Free Distilled Water	500 mL	10977-015

Technical Support

Obtaining Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

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

Certificate of Analysis

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

Technical Support, Continued

Limited Product Warranty

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

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Notes

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