invitrogen

# PureLink™ Pro 96 RNA Purification Kit

## USER GUIDE

For high-throughput purification of total RNA

**Catalog Number** 12173–011A

**Publication Number** MAN0001670

**Revision** C.0

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

ThermoFisher SCIENTIFIC

NOTE TO WRITER: ADD CONKEYREF SOURCE TO PUBLICATION

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**Revision history:** Revision history of Pub. no. MAN0001670

| Revision | Date | Description |
|---|---|---|
| C.0 | 20 September 2017 | Removal of redundant step in "Prepare mammalian cell lysates" |
| B.0 | 06 December 2016 | Correction of volumes of the kit contents |
| A.0 | 01 April 2016 | Removed the link to the methods for preparing workstation decks of automated liquid handling |
| 4.0 | 27 September 2012 | Baseline for revisions |



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# Contents

# Product information

**IMPORTANT!** Before using this product, read and understand the information in the "Safety" appendix in this document.

## Product description

The Invitrogen™ PureLink™ Pro 96 RNA Purification Kit allows for high-throughput isolation of total RNA from 96 different cell samples.The kit uses silica-based membranes to bind the RNA, ensuring high yield and purity and minimal genomic DNA contamination. Total RNA isolated using the PureLink™ Pro 96 RNA Purification Kit is suitable for use in Northern blotting, nuclease protection assays, reverse transcriptions, RT-PCR, real-time quantitative PCR (qPCR).

The PureLink™ Pro 96 RNA Purification Kit is designed for use with a vacuum manifold or a centrifuge and is compatible with most automated liquid handling. This document described RNA isolation from bacteria, yeast, plant, mammalian cells, tissues, and virus.

## Contents and storage

Sufficient reagents are included in the kit to perform 384 (4 × 96) isolations.

**Table 1** PureLink™ Pro 96 RNA Purification Kit (Cat. No. 12173–011A)

| Contents | Amount | Storage |
|---|---|---|
| PureLink™ Pro 96 Lysis Buffer | 150 mL | 15°C to 30°C |
| PureLink™ Pro 96 Wash Buffer I | 250 mL | |
| PureLink™ Pro 96 Wash Buffer II (5X) | 250 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 | |

# Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

| Item | Source |
|---|---|
| **Equipment** | |
| Benchtop microcentrifuge | MLS |
| Centrifuge with a swinging bucket rotor with plate carries that have a plate height clearance of 7.0 cm (for isolation using centrifugation only) | MLS |
| Vacuum manifold and vacuum pump (producing pressure of 12–15 in. Hg) or automated liquid handling workstation (for isolation using vacuum manifold only) | MLS |
| Laboratory mixer (Vortex or equivalent) | MLS |
| Homogenizer or tissue grinder (for plant and tissue lysates only) | MLS |
| **Tubes, plates, and accessories** | |
| Disposable, individually wrapped, sterile plasticware | MLS |
| Microcentrifuge tube, 1.5 mL | MLS |
| Multichannel pipettes | MLS |
| Aerosol-resistant pipette tips | MLS |
| PureLink™ 96 Receiver Plates | 12193025 |
| **Reagents** | |
| Ethanol, 75% | MLS |
| Ethanol, 95–100% | MLS |
| TE Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) | MLS |
| *(Optional)* β-Mercaptoethanol | MLS |
| *(Optional)* DNase I solution | MLS |
| Lysozyme (for bacterial lysate only) | MLS |
| 5% SDS solution (for bacterial lysate only) | MLS |
| Zymolase or lyticase (for yeast lysate only) | MLS |

# Methods

## Procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- 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*™ Decontamination Reagent (Cat. No. 10328011) or RNase*Zap*™ RNase Decontamination Solution (Cat. No. AM9780) to remove RNase contamination from surfaces and from non-disposable equipment like homogenizers.
- Do not store lysates in PureLink™ Pro 96 Lysis Buffer. Isolate RNA immediately after lysing samples to avoid RNA degradation.

## Before you begin

- Add 10 µL of 14.3 M β-mercaptoethanol to 1 mL of PureLink™ Pro 96 Lysis Buffer before lysing the samples.
- **For bacterial samples:** Prepare 1% lysozyme in TE Buffer and sterilize by filtration the solution.
- **For isolation using a centrifuge:** Prepare 150 mL of 1X PureLink™ Pro 96 Wash Buffer II for one 96-well plate as indicated in the following table.

| Reagents | Quantity |
|---|---|
| PureLink™ Pro 96 Wash Buffer II (5X) | 30 mL |
| Ethanol, 95–100% | 120 mL |
| **Total volume** | 150 mL |

- **For isolation using a vacuum manifold:** Prepare 200 mL of 1X PureLink™ Pro 96 Wash Buffer II for one 96-well plate as indicated in the following table.

| Reagents | Quantity |
|---|---|
| PureLink™ Pro 96 Wash Buffer II (5X) | 40 mL |
| Ethanol, 95–100% | 160 mL |
| **Total volume** | 200 mL |

# Prepare lysates

This section describes protocols to prepare lysates from bacteria (see page 8), yeast (see page 8), plant (see page 8), mammalian cells (see page 9), and tissues (see page 9) and to clean up samples after TRIzol™ extraction (see page 10). To prepare cells to isolate viral RNA, follow the mammalian cell lysate protocol (see page 9).

## Prepare bacterial lysates

1. Harvest up to 1 x $10^9$ *E. coli* cells by centrifugation.
2. Resuspend the pellet in 43 µL of 1% lysozyme in TE Buffer.
3. Add 1 µL of 5% SDS to the lysate and mix thoroughly.
4. Incubate for 5 minutes.
5. Add 150 µL of PureLink™ Pro 96 Lysis Buffer and 106 µL of 100% ethanol.
6. Mix thoroughly.

Proceed immediately to "Isolate RNA using centrifugation" on page 10 or "Isolate RNA using vacuum manifold" on page 11.

## Prepare yeast lysates

1. Harvest 1.8 mL of 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 (or lyticase).
4. Incubate for 30 minutes.
5. Add 150 µL of PureLink™ Pro 96 Lysis Buffer and 107 µL of 100% ethanol.
6. Mix thoroughly.

Proceed immediately to "Isolate RNA using centrifugation" on page 10 or "Isolate RNA using vacuum manifold" on page 11.

## Prepare plant lysates

1. Prepare plant tissue:
   - **Hard tissue:** Freeze up to 150 mg of hard plant tissue in liquid nitrogen and grind to powder.
   - **Soft tissue:** Cut soft, non-fibrous plant tissue into small pieces.
2. Prepare lysate.
   - **Hard tissue:** Vortex the ground tissue for 1 minute.
   - **Soft tissue:** Homogenize with a homogenizer or tissue grinder.
3. Centrifuge the lysate at high speed to remove insoluble materials.
4. Add 150 µL of 70 ethanol and mix thoroughly.

Proceed immediately to "Isolate RNA using centrifugation" on page 10 or "Isolate RNA using vacuum manifold" on page 11.

## Prepare mammalian cell lysates

1. Grow up to $5 \times 10^5$ cells in regular 96-well culture plates (~300 µL capacity).
   For cell numbers greater than $5 \times 10^5$, use 96 deep-well plates (~1 mL capacity).
2. Harvest the cells.
   - **Adherent cells:** Remove the growth medium from the culture wells.
   - **Suspension cells:** Centrifuge the culture plate at 250 × *g* for 5 minutes, then remove the growth medium.
3. Add PureLink™ Pro 96 Lysis Buffer.
   - **For <5 × $10^5$ cells:** Add 150 µL of PureLink™ Pro 96 Lysis Buffer and 150 µL of 70% ethanol.
   - **For >5 × $10^5$ cells:** Add 350 µL of PureLink™ Pro 96 Lysis Buffer and 350 µL of 70% ethanol.
4. Mix thoroughly by pipetting up and down.

Proceed immediately to "Isolate RNA using centrifugation" on page 10 or "Isolate RNA using vacuum manifold" on page 11.

## Prepare tissue lysates

1. Place ~100 mg of minced mammalian tissue in a 15-mL disposable tube.
2. Add 4.8 mL of PureLink™ Pro 96 Lysis Buffer
3. Homogenize the tissue.
4. Centrifuge the lysate for 10 minutes at 2600 × *g*.
5. Transfer the supernatant to a fresh tube.
6. Add 4.8 mL of 70% ethanol.
7. Mix thoroughly.

Proceed immediately to "Isolate RNA using centrifugation" on page 10 or "Isolate RNA using vacuum manifold" on page 11. Use 500 µL–900 µL(5–9 mg of the tissue lysate per well.

**Note:** The amount of lysate depends on the tissue type. Use less viscous lysates or lysates containing high lipid content, as they may clog the PureLink™ 96 Well Total RNA Filter Plate.

### Prepare samples after TRIzol™ extraction

1. Collect the upper colorless aqueous phase (containing the RNA) after performing phase separation during the TRIzol™ extraction.
2. Add a volume of PureLink™ Pro 96 Lysis Buffer equal to the volume of the collected aqueous phase.
3. Add a volume of 100% ethanol equal to the volume of the collected aqueous phase.
4. Mix thoroughly.

Proceed immediately to "Isolate RNA using centrifugation" on page 10 or "Isolate RNA using vacuum manifold" on page 11.

## Isolate RNA using centrifugation

### Bind the RNA to the membrane

1. Place a PureLink™ 96 Well Total RNA Filter Plate on top of a PureLink™ 96 Receiver Plate.
2. Transfer lysate to the wells of the PureLink™ 96 Well Total RNA Filter Plate.
3. Centrifuge the stacked plates for 1–2 minutes at ≥2100 × *g*.
4. Remove the PureLink™ 96 Well Total RNA Filter Plate from the top of the PureLink™ 96 Receiver Plate.
5. Discard flow-through from the PureLink™ 96 Receiver Plate.
6. Replace the PureLink™ 96 Well Total RNA Filter Plate on top of the PureLink™ 96 Receiver Plate.

### Wash the RNA on the membrane

1. Add 500 µL of PureLink™ Pro 96 Wash Buffer I to the sample wells of the PureLink™ 96 Well Total RNA Filter Plates.
2. Centrifuge the stacked plates for 1–2 minutes at ≥2100 × *g*.
3. Remove the PureLink™ 96 Well Total RNA Filter Plate from the top of the PureLink™ 96 Receiver Plate.
4. Discard flow-through from the PureLink™ 96 Receiver Plate.
5. Replace the PureLink™ 96 Well Total RNA Filter Plate on top of the PureLink™ 96 Receiver Plate.
6. *(Optional)* Remove genomic DNA by performing an on-column DNase digestion (see Appendix C, "On-column DNase digestion").
7. Add 750 µL of 1X PureLink™ Pro 96 Wash Buffer II to the sample wells of the PureLink™ 96 Well Total RNA Filter Plates.
8. Centrifuge the stacked plates for 1–2 minutes at ≥2100 × *g*.

9. Remove the PureLink™ 96 Well Total RNA Filter Plate from the top of the PureLink™ 96 Receiver Plate.
10. Discard flow-through from the PureLink™ 96 Receiver Plate.
11. Replace the PureLink™ 96 Well Total RNA Filter Plate on top of the PureLink™ 96 Receiver Plate.
12. Repeat step 7–step 11.
13. Centrifuge the stacked plates for 10 minutes at ≥2100 × *g* to dry the membrane.

### Elute the RNA

1. Place a PureLink™ 96 Well Total RNA Filter Plate on top of a PureLink™ 96 Elution Plate.
2. Add 45 µL of PureLink™ Pro 96 RNase-free Water to the sample wells of the PureLink™ 96 Well Total RNA Filter Plates.
3. Incubate for 1 minute.
4. Centrifuge the stacked plates for 1–2 minutes at ≥2100 × *g*.
   The RNA is in the wells of the PureLink™ 96 Elution Plate.

Store the RNA in the PureLink™ 96 Elution Plate for immediate use or transfer to RNase-free tubes for longer-term storage.

## Isolate RNA using vacuum manifold

### Bind the RNA to the membrane

1. Prepare the vacuum manifold, or the automated liquid handler, according to the manufacturer's recommendations.
2. Place a PureLink™ 96 Well Total RNA Filter Plate on the vacuum manifold.
3. Transfer lysate to the wells of the PureLink™ 96 Well Total RNA Filter Plate.
4. Apply vacuum for 2 minutes.

### Wash the RNA on the membrane

1. Add 500 µL of PureLink™ Pro 96 Wash Buffer I to the sample wells of the PureLink™ 96 Well Total RNA Filter Plates.
2. Apply vacuum for 2 minutes.
3. *(Optional)* Remove genomic DNA by performing an on-column DNase digestion (see Appendix C, “On-column DNase digestion“).
4. Add 1 mL of 1X PureLink™ Pro 96 Wash Buffer II to the sample wells of the PureLink™ 96 Well Total RNA Filter Plates.
5. Apply vacuum for 2 minutes.
6. Repeat step 4–step 5.

7. Pat firmly the PureLink™ 96 Well Total RNA Filter Plate with the filter side down on a stack of paper towels to blot residual liquid.
8. Place the PureLink™ 96 Well Total RNA Filter Plate on the vacuum manifold.
9. Apply vacuum for 5–10 minutes.

### Elute the RNA

1. Place a PureLink™ 96 Elution Plate in the vacuum manifold in place of the waster collection tray.
2. Place the PureLink™ 96 Well Total RNA Filter Plate on top of the PureLink™ 96 Elution Plate.
3. Add 170 µL of PureLink™ Pro 96 RNase-free Water to the sample wells of the PureLink™ 96 Well Total RNA Filter Plates.
4. Incubate for 1 minute.
5. Apply vacuum for 2 minutes.
   The RNA is in the wells of the PureLink™ 96 Elution Plate.

Store the RNA in the PureLink™ 96 Elution Plate for immediate use or transfer to RNase-free tubes for longer-term storage.

## Determine RNA quality and quantity

### Estimate 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.
2. Mix well, then transferred to a cuvette (1-cm path length).

   **Note:** The RNA must be in a neutral buffer to accurately measure the UV absorbance.
3. Determine the $OD_{260}$ of the solution using a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.0.
4. Calculate the amount of total RNA using the following formula:

   Total RNA (µg) = $OD_{260}$ × [40 µg/(1 $OD_{260}$ × 1 mL)] × dilution factor × 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 7.0. An $OD_{260}$ of 0.188 was obtained. The amount of RNA in the sample is calculated as follow:

   Total RNA (µg) = 0.188 × [40 µg/(1 $OD_{260}$ × 1 mL)] × 12.5 × 0.15 = 14.1 µg of total RNA

## Analysis of the 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 $OD_{260/A280}$ 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 in the following table.

**Table 2** Sizes of ribosomal RNA from various sources

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

# Troubleshooting

| Observation | Possible cause | Recommended action |
|---|---|---|
| Low RNA yield | The lysis is incomplete 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. |
| | | Add ß-mercaptoethanol to PureLink™ Pro 96 Lysis Buffer to improve cell lysis. |
| RNA degraded | The RNA is contaminated with RNase. | Follow "Procedural guidelines" on page 7. |
| Genomic DNA contamination | The RNA is contaminated with genomic DNA. | Perform DNase I digestion to remove genomic DNA contamination (see Appendix C, "On-column DNase digestion") . |
| Low elution volume or sample cross-contamination<br>(For isolation using vacuum manifold only) | Incorrect vacuum pressure was applied. | Make sure the vacuum manifold is sealed tightly and there is no leakage.<br>A vacuum pressure of 12-15 in. Hg is required to obtain the best results. |

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# Expected results

## RNA yield

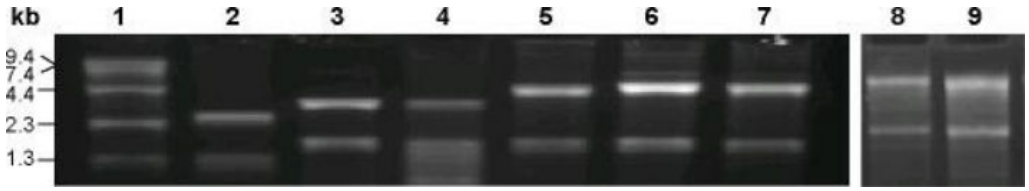
The yield of total RNA obtained from various sources is listed in the following table.

**Table 3** Yield of total RNA obtained from various sources

| Source | Amount | Total RNA yield |
|---|---|---|
| Bacteria (*E. coli*) | $5 \times 10^8$ cells | 3.74 µg |
| Yeast (*S. cerevisae*) | $2 \times 10^8$ cells | 33.59 µg |
| Plant (lettuce) | 100 mg | 10.33 µg |
| **Mammalian cells:** | | |
| Mouce NIH3T3 cells | $3.7 \times 10^5$ cells | 14.38 µg |
| Human 293 cells | $3.7 \times 10^5$ cells | 16.91 µg |
| Human HeLa cells | $3.7 \times 10^5$ cells | 9.56 µg |
| **Tissues:** | | |
| Mouse brain | 9 mg | 5.4 µg |
| Mouse liver | 5 mg | 18.2 µg |

# RNA integrity

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




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

# 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 (Cat. No. 11731015).

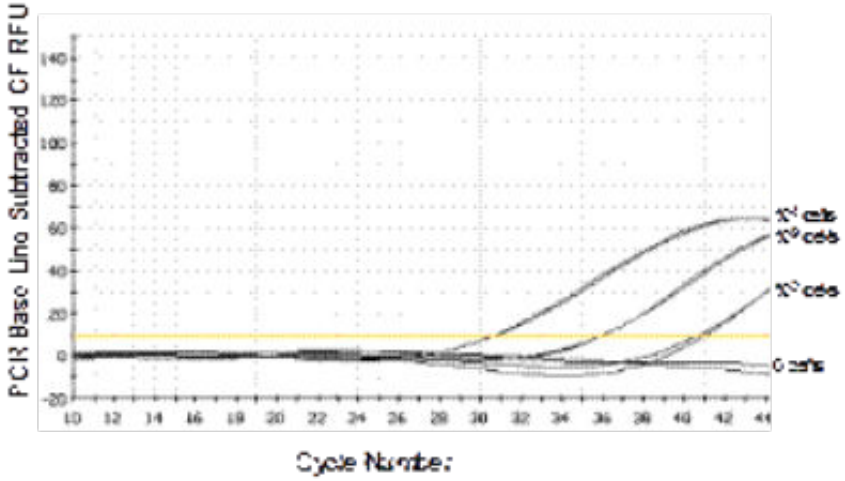



**Figure 1** Gene-specific real time RT-PCR of RNA

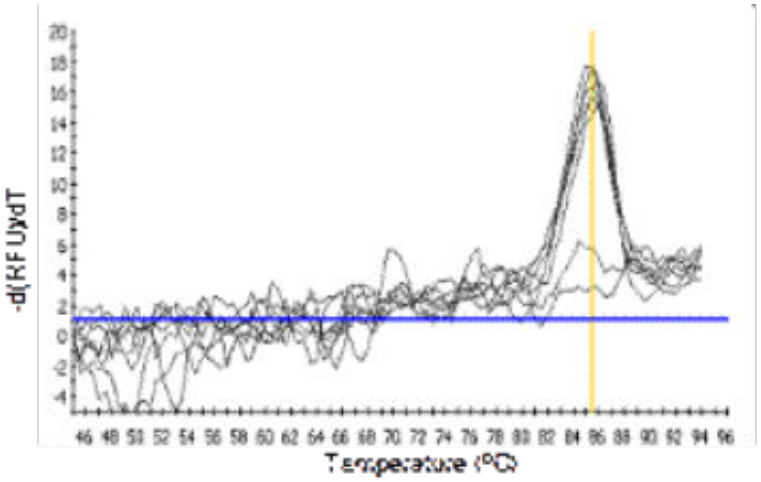



**Figure 2** Melting temperature analysis of amplified hSDHA PCR product

C

# On-column DNase digestion

## Prepare DNase I solution

1. Prepare the 10X DNase I Buffer as indicated in the following table.

| Reagents | Quantity |
|---|---|
| 1M Tris-HCl, pH 8.4 | 200 µL |
| 100 mM $MgCl_2$ | 200 µL |
| 1M KCl | 500 µL |
| RNase-free Water | to 8 mL |
| **Total volume** | 8 mL |

**Note:** If you are using DNase I, Amplification Grade (Cat. No. 18068–015), there is no need to prepare the 10X DNase Buffer. This enzyme is provided with a vial of 10X DNase I reaction buffer).

2. Prepare 8 mL of DNase I Solution for each well as indicated in the following table and mix well.

   Always use freshly prepared DNase I Solution

| Reagents | Quantity |
|---|---|
| 10X DNase I Buffer | 0.8 mL |
| DNase I | 3200 units |
| RNase-free Water | to 8 mL |
| **Total volume** | 8 mL |

**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

## Digest with DNase I - Isolation using centrifugation

1. Add 80 µL of DNase I Solution to each well of the PureLink™ 96 Well Total RNA Filter Plate .
2. Incubate for 15 minutes.
3. Add 500 µL of PureLink™ Pro 96 Washer Buffer I.
4. Incubate for 5 minutes.
5. Centrifuge the stacked plates for 1–2 minutes at ≥2100 × *g*.
6. Remove the PureLink™ 96 Well Total RNA Filter Plate from the top of the PureLink™ 96 Receiver Plate.
7. Discard flow-through from the PureLink™ 96 Receiver Plate.
8. Replace the PureLink™ 96 Well Total RNA Filter Plate on top of the PureLink™ 96 Receiver Plate.

Proceed with the end of the protocol normally.

## Digest with DNase I - Isolation using a vacuum manifold

1. Add 80 µL of DNase I Solution to each well of the PureLink™ 96 Well Total RNA Filter Plate .
2. Apply vacuum briefly to allow the solution to soak into the membrane.
3. Incubate for 15 minutes
4. Add 500 µL of PureLink™ Pro 96 Washer Buffer I.
5. Incubate for 5 minutes.
6. Apply vacuum for 2 minutes.

Proceed with the end of the protocol normally.

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# Isolate RNA using the EveryPrep™ Universal Vacuum Manifold

## Before you begin

- Add 10 µL of 14.3 M β-mercaptoethanol to 1 mL of PureLink™ Pro 96 Lysis Buffer before lysing the samples.
- **For bacterial samples:** Prepare 1% lysozyme in TE Buffer and sterilize by filtration the solution.
- **For isolation using a centrifuge:** Prepare 150 mL of 1X PureLink™ Pro 96 Wash Buffer II for one 96-well plate as indicated in the following table.

| Reagents | Quantity |
|---|---|
| PureLink™ Pro 96 Wash Buffer II (5X) | 30 mL |
| Ethanol, 95–100% | 120 mL |
| **Total volume** | 150 mL |

- **For isolation using a vacuum manifold:** Prepare 200 mL of 1X PureLink™ Pro 96 Wash Buffer II for one 96-well plate as indicated in the following table.

| Reagents | Quantity |
|---|---|
| PureLink™ Pro 96 Wash Buffer II (5X) | 40 mL |
| Ethanol, 95–100% | 160 mL |
| **Total volume** | 200 mL |

## Bind the RNA to the membrane

1. Prepare the EveryPrep™ Universal Vacuum Manifold according to the manufacturer's recommendations.
2. Place a PureLink™ 96 Well Total RNA Filter Plate on the EveryPrep™ Universal Vacuum Manifold.
3. Transfer lysate to the wells of the PureLink™ 96 Well Total RNA Filter Plate.
4. Apply vacuum for 2 minutes.

## Wash the RNA on the membrane

1. Add 500 µL of PureLink™ Pro 96 Wash Buffer I to the sample wells of the PureLink™ 96 Well Total RNA Filter Plates.
2. Apply vacuum for 2 minutes.
3. *(Optional)* Remove genomic DNA by performing an on-column DNase digestion (see Appendix C, "On-column DNase digestion").
4. Add 1 mL of 1X PureLink™ Pro 96 Wash Buffer II to the sample wells of the PureLink™ 96 Well Total RNA Filter Plates.
5. Apply vacuum for 2 minutes.
6. Repeat step 4–step 5.
7. Pat firmly the PureLink™ 96 Well Total RNA Filter Plate with the filter side down on a stack of paper towels to blot residual liquid.
8. Place the PureLink™ 96 Well Total RNA Filter Plate on the vacuum manifold.
9. Apply vacuum for 5 minutes.

## Elute RNA

1. Place the Elution Block and a PureLink™ 96 Elution Plate in the Elution Chamber and cover the top with the 96 Well Top Plate.
2. Place the PureLink™ 96 Well Total RNA Filter Plate over the Top Plate.
3. Add 170 µL of PureLink™ Pro 96 RNase-free Water to the sample wells of the PureLink™ 96 Well Total RNA Filter Plates.
4. Incubate for 1 minute.
5. Apply vacuum for 2 minutes.
   The RNA is in the wells of the PureLink™ 96 Elution Plate.

Store the RNA in the PureLink™ 96 Elution Plate for immediate use or transfer to RNase-free tubes for longer-term storage.

# E Ordering information

## Additional products

The following products are also available through **thermofisher.com**.

| Item | Quantity | Cat. No. |
|---|---|---|
| **PureLink™ 96 RNA components available separately** | | |
| PureLink™ 96 Receiver Plates (deep-well) | 50 plates | 12193025 |
| PureLink™ 96 RNA Lysis Buffer | 750 mL | 12173022 |
| PureLink™ 96 RNA Wash Buffer I (1X) | 5 × 1 L | 12173032 |
| PureLink™ 96 RNA Wash Buffer II (5X) | 2 × 1 L | 12173033 |
| PureLink™ RNA Mini Kit | 50 reactions | 12183018A |
| | 10 reactions | 12183020 |
| **Vacuum manifold** | | |
| EveryPrep™ Universal Vacuum Manifold | 1 manifold | K211101 |
| **Reagents for RT-PCR** | | |
| SuperScript™ One-Step RT-PCR System with Platinum™ *Taq* DNA Polymerase | 100 reactions | 10928042 |
| Platinum™ Quantitative RT-PCR ThermoScript™ One-Step System | 100 reactions | 11731015 |
| **Reagents** | | |
| RNase *AWAY*™ Decontamination Reagent | 250 mL | 10328011 |
| RNase*Zap*™ RNase Decontamination Solution | 250 mL | AM9780 |
| DNase I | 20,000 units | 18047019 |
| DNase I, Amplification Grade | 100 unites | 18068015 |
| 0.1–2 kb RNA Ladder | 75 µg | 15623100 |
| 0.5–10 kb RNA Ladder | 75 µg | 15623200 |
| UltraPure™ DEPC-treated Water | 1 L | 750023 |
| UltraPure™ DNase/RNase-Free Distilled Water | 500 mL | 10977-015 |

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# Safety

**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.

# 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. 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.

# Biological hazard safety

**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. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  **www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf**
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  **www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf**

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    **Note:** For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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