

PureLink™ Pro 96 RNA Purification Kit

USER GUIDE

For high-throughput purification of total RNA

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D.0	15 December 2022	Minor revisions and corrections made throughout manual.
C.0	20 September 2017	Removal of redundant step in "Prepare mammalian cell lysates".
B.0	6 December 2016	Correction of volumes of the kit contents.
A.0	1 April 2016	Removed the link to the methods for preparing workstation decks of automated liquid handling.
A.0	27 September 2012	Baseline for revisions.

The information in this guide is subject to change without notice.

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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. [12173011A](#))

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" indicates that the material is available from fisherscientific.com or another 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
<i>(Optional)</i> β-Mercaptoethanol	MLS
<i>(Optional)</i> 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

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 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 RNaseZap™ 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.
- **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

- 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 1×10^9 *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 at room temperature.
5. Add 150 μL of PureLink™ Pro 96 Lysis Buffer (supplemented with β -mercaptoethanol) 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 12.

Prepare yeast lysates

1. Harvest 1.8 mL of fresh, log-phase yeast cells ($\text{OD}_{660}=0.6\text{--}0.8$) 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 at room temperature.
5. Add 150 μL of PureLink™ Pro 96 Lysis Buffer (supplemented with β -mercaptoethanol) 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 12.

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. Add 150 μL PureLink™ Pro 96 Lysis Buffer (supplemented with β -mercaptoethanol) to the tissue and proceed according to the tissue-type-specific instructions below.
 - **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 to 150 μL plant lysate, then mix thoroughly.

Proceed immediately to “Isolate RNA using centrifugation” on page 10 or “Isolate RNA using vacuum manifold” on page 12.

Prepare mammalian cell lysates

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. Harvest the cells.
 - **Adherent cells:** Remove the growth medium from the culture wells.
 - **Suspension cells:** Centrifuge the culture plate at $250 \times g$ for 5 minutes, then remove the growth medium.
3. Add PureLink™ Pro 96 Lysis Buffer.
 - **For $<5 \times 10^5$ cells:** Add 150 μL of PureLink™ Pro 96 Lysis Buffer (supplemented with β -mercaptoethanol) and 150 μL of 70% ethanol.
 - **For $>5 \times 10^5$ cells:** Add 350 μL of PureLink™ Pro 96 Lysis Buffer (supplemented with β -mercaptoethanol) 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 12.

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 at $2600 \times g$ for 10 minutes at room temperature.
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 12. Use 500 μ L–900 μ L (5–9 mg of the tissue lysate per well).

Note: Viscous lysates or lysates containing high lipid content may clog the PureLink™ 96 Well Total RNA Filter Plate. Use a smaller volume of lysate for these sample types and scale back the amount of starting tissue for future extractions.

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 12.

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 $\geq 2100 \times g$.
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 at $\geq 2100 \times g$ for 1–2 minutes.
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 (diluted in ethanol) to the sample wells of the PureLink™ 96 Well Total RNA Filter Plates.
8. Centrifuge the stacked plates at $\geq 2100 \times g$ for 1–2 minutes.
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 $\geq 2100 \times g$ 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 at room temperature for one minute.
4. Centrifuge the stacked plates at $\geq 2100 \times g$ for 1–2 minutes.
The RNA is in the wells of the PureLink™ 96 Elution Plate.

Use the RNA immediately or transfer to RNase-free tubes for long-term storage at -80°C .

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.

Note: If using the EveryPrep™ Universal Vacuum Manifold (Cat. No. [K211101](#)), follow the protocol provided in Appendix D (see “Bind the RNA to the membrane” on page 20).

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, then release.

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, then release.
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 (with added ethanol) to the sample wells of the PureLink™ 96 Well Total RNA Filter Plates.
5. Apply vacuum for 2 minutes, then release.
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 to dry.

Elute the RNA

1. Place a PureLink™ 96 Elution Plate in the vacuum manifold in place of the waste 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 at room temperature.
5. Apply vacuum for 2 minutes, then release.
The RNA is in the wells of the PureLink™ 96 Elution Plate.

Use the RNA immediately or transfer to RNase-free tubes for long-term storage at -80°C.

Determine RNA quality and quantity

Estimate RNA quantity

Use a spectrophotometer to determine the quantity of the purified total RNA by UV absorbance at OD₂₆₀.

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 OD₂₆₀ 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:

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

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 7.0. An OD₂₆₀ of 0.188 was obtained. The amount of RNA in the sample is calculated as follow:

$$\text{Total RNA } (\mu\text{g}) = 0.188 \times [40 \mu\text{g}/(1 \text{ OD}_{260} \times 1 \text{ mL})] \times 12.5 \times 0.15 = 14.1 \mu\text{g of total RNA}$$

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, chlorophyll, 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 discrete 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 or 18S	23S or 28S
<i>E. coli</i>	1.5 kb	2.9 kb
<i>S. cerevisiae</i>	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 $>5 \times 10^5$ to achieve complete lysis.
		Add β -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 Details: (For isolation using vacuum manifold only)	Incorrect vacuum pressure was applied.	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.



Expected results

RNA yield

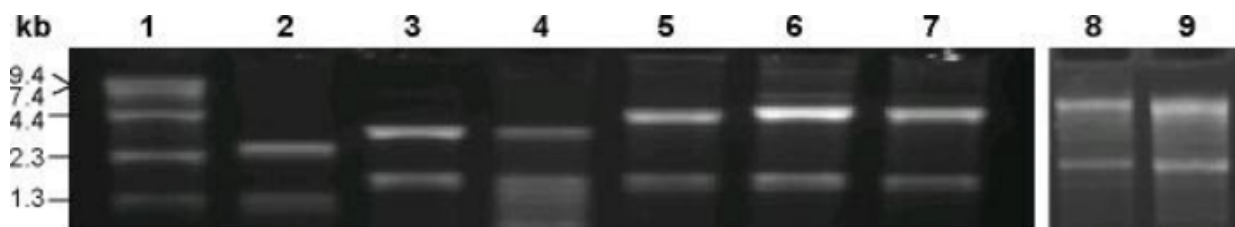
The yield of total RNA obtained from various sources is listed in the following table.

Table 3 Yield of total RNA obtained from various sources

Source	Amount	Total RNA yield
Bacteria (<i>E. coli</i>)	5×10^8 cells	3.74 μg
Yeast (<i>S. cerevisiae</i>)	2×10^8 cells	33.59 μg
Plant (lettuce)	100 mg	10.33 μg
Mammalian cells:		
Mouce NIH3T3 cells	3.7×10^5 cells	14.38 μg
Human 293 cells	3.7×10^5 cells	16.91 μg
Human HeLa cells	3.7×10^5 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 1.0 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



On-column DNase digestion

Prepare DNase I solution

1. Prepare DNase I Solution for each well as indicated in the following table and mix well.

Note: Always use freshly prepared DNase I Solution.

Reagents	Quantity
10X DNase I Buffer	0.8 mL
DNase I ^[1]	3200 units
RNase-free Water	to 8 mL
Total volume	8 mL

^[1] We recommend the use of PureLink™ DNase Set (Cat. No. [12185010](#)), which has been specifically formulated for PureLink™ on-column DNA 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

2. If the DNase enzyme did not come with a 10X Buffer, 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



Digest with DNase I - Isolation using centrifugation

1. Add 80 μ L of DNase I Solution (see “Prepare DNase I solution” on page 18) to each well of the PureLink™ 96 Well Total RNA Filter Plate .
2. Incubate for 15 minutes at room temperature.
3. Add 500 μ L of PureLink™ Pro 96 Washer Buffer I.
4. Incubate for 5 minutes at room temperature.
5. Centrifuge the stacked plates at $\geq 2100 \times g$ for 1–2 minutes.
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 rest of the protocol as described (see “Wash the RNA on the membrane” on page 11).

Digest with DNase I - Isolation using a vacuum manifold

1. Add 80 μ L 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 at room temperature.
4. Add 500 μ L of PureLink™ Pro 96 Washer Buffer I.
5. Incubate for 5 minutes at room temperature.
6. Apply vacuum for 2 minutes, then release.

Proceed with the rest of the protocol as described (see “Wash the RNA on the membrane” on page 12).



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.
- 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 (Cat. No. [K211101](#)) 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, then release.
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 which ethanol has been added) to the sample wells of the PureLink™ 96 Well Total RNA Filter Plates.
5. Apply vacuum for 2 minutes, then release.
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, to dry.

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, then release.
The RNA is in the wells of the PureLink™ 96 Elution Plate.

Use the RNA immediately or transfer to RNase-free tubes for long-term storage at -80°C.



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
RNaseZap™ RNase Decontamination Solution	250 mL	AM9780
PureLink™ DNase Set	50 preparations	12185010
Ambion™ DNase I (RNase-free)	5 × 2,000 units	AM2224
Ambion™ DNase I (RNase-free)	2,000 units	AM2222
DNase I, Amplification Grade	100 units	18068015
Century-Plus RNA Markers, 0.1–1.0 kb Ladder	50 µg	AM7145
Millenium RNA Markers	50 µg	AM7150

(continued)

Item	Quantity	Cat. No.
UltraPure™ DEPC-treated Water	1 L	750023
UltraPure™ DNase/RNase-Free Distilled Water	500 mL	10977015



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, and so on). To obtain SDSs, visit [thermofisher.com/support](https://www.thermofisher.com/support).

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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
www.who.int/publications/i/item/9789240011311



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