invitrogen

PureLink™ miRNA Isolation Kit

USER GUIDE

For rapid, efficient purification of small RNA molecules

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Revision	Date	Description
A.0	13 December 2016	Updated to latest branding guidelines.
2.0	27 September 2012	Baseline for revisions.

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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™ miRNA Isolation Kit allows for isolation of small RNA molecules from biological samples for functional analysis. The kit is specifically designed to isolate high-quality small RNA molecules including tRNA, 5S rRNA, 5.8S rRNA, and regulatory RNA molecules such as microRNA (miRNA) and short interfering RNA (siRNA). PureLink™ miRNA Isolation Kit uses silica-based membranes to bind the RNA, ensuring high yield and purity and minimal genomic DNA contamination. The isolated RNA using the PureLink™ miRNA Isolation Kit is suitable for use in Northern blotting and microarray analysis.

This document described RNA isolation from bacteria, yeast, plant, mammalian cells, tissues, and virus.

Content and storage

Sufficient reagents are included in the kit to perform 25 reactions.

Table 1 PureLink™ miRNA Isolation Kit (Cat. No. K157001)

Contents	Amount	Storage
Binding Buffer (L3)	45 mL	15°C to 30°C
Wash Buffer (W5)	10 mL	
Buffer (W4) for single column purification	3 mL	
Sterile, RNase-free Water	5 mL	
Spin Cartridges with Collection Tubes	50 cartridges	
Wash Tubes (2 mL)	50 tubes	
Recovery Tubes (1.7 mL)	50 tubes	

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
Mircocentrifuge capable of reaching >10,000 × *g*	MLS
Laboratory mixer (vortex or equivalent)	MLS
Homogenizer (for viscous or debris-containing samples only)	12183026
Homogenizer or tissue grinder (for plant and tissue lysates only)	MLS
Water bath or heat block at 30°C (for yeast lysate only)	
Tubes, plates, and accessories	
Disposable, individually wrapped, sterile plasticware	MLS
Sterile, DNase-free microcentrifuge tubes, 1.5 mL	MLS
Multichannel pipettes	MLS
Aerosol-resistant pipette tips	MLS
Reagents	
Ethanol, 70%	MLS
Ethanol, 96–100%	MLS
TE Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)	MLS
Lysozyme (for bacterial lysate only)	MLS
5% SDS solution (for bacterial lysate only)	MLS
Zymolase or lyticase (for yeast lysate only)	MLS
TRIzol™ Reagent (for large sample amount only)	15596026
Chloroform (for large sample amount only)	MLS
UltraPure™ Glycogen, 20 µg/µL (for one-column protocol only)	10814-010

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.

Before you begin

- **For bacterial samples:** Prepare 1% lysozyme in TE Buffer and sterilize by filtration the solution.
- Add 40 mL of 96–100% ethanol to 10 mL of Wash Buffer (W5), then store at room temperature.
- **For one-column protocol:** Add 1.5 mL of RNase-free Water and 1.5 mL of 96–100% ethanol to 10 mL of Wash Buffer (W4), then store at room temperature.

Prepare lysates

This section describes protocols to prepare lysates from:

Prepare mammalian cell lysates

1. Harvest the cells.
   - **Adherent cells** (up to 1×10^6 cells): Remove the growth medium from the culture wells.
   - **Suspension cells** (up to 1×10^6 cells): Centrifuge the culture plate at 250 × *g* for 5 minutes, then remove the growth medium.
2. Add 300 µL of Binding Buffer (L3).
3. Mix well by vortexing or pipetting up and down.
4. Add 300 µL of 70% ethanol.
5. Mix well by vortexing.

Proceed immediately to "Isolate RNA" on page 11.

Prepare tissue lysates

1. Place ~5 mg of minced mammalian tissue in a sterile microcentrifuge tube.
2. Add 300 µL of Binding Buffer (L3).
   Ensure that the tissue is completely immersed in the buffer.
3. Homogenize the tissue using a tissue homogenizer.
4. Centrifuge the lysate for 5 minutes at 12,000 × *g*.
5. Transfer the supernatant to a fresh tube.

   Note: If the lysate is viscous or contains cell debris, clarify the lysate using the Homogenizer (Cat. No. 12183026).
6. Add 300 µL of 70% ethanol.
7. Mix well by vortexing.

Proceed immediately to "Isolate RNA" on page 11.

Prepare plant lysates

1. Prepare plant tissue:
   - **Hard tissue:** Freeze the plant tissue in liquid nitrogen and grind to a powder.
   - **Soft tissue:** Cut soft, non-fibrous plant tissue into small pieces.
2. Add 300 µL of Binding Buffer (L3).
3. Prepare lysate.
   - **Hard tissue:** Vortex the ground tissue until the powder is completely resuspended.
   - **Soft tissue:** Homogenize with a homogenizer or tissue grinder.
4. Centrifuge the lysate at 12,000 × *g* for 2 minutes to remove insoluble materials.

5. Transfer the supernatant to a new microcentrifuge tube.

   Note: If the lysate is viscous or contains cell debris, clarify the lysate using the Homogenizer (Cat. No. 12183026).

6. Add 300 µL of 70% ethanol.

7. Mix well by vortexing.

Proceed immediately to "Isolate RNA" on page 11.

Prepare yeast lysates

If you are using a frozen cell pellet, proceed directly to step 2.

1. Harvest 1 mL of fresh, log-phase yeast cells (OD_{660}=1.04) by centrifugation.
2. Resuspend the pellet in 70 µL of cold TE Buffer.
3. Add 30 units of zymolase (or lyticase).
4. Centrifuge the lysate at 12,000 × *g* for 2 minutes to remove insoluble materials.
5. Resuspend the pellet in 300 µL of Binding Buffer (L3).
6. Add 210 µL of 96-100 ethanol.
7. Mix well by vortexing.

Proceed immediately to "Isolate RNA" on page 11.

Prepare bacterial lysates

If you are using a frozen cell pellet, proceed directly to step 2.

1. Harvest up to 5 x 10^6 *E. coli* cells by centrifugation.
2. Resuspend the pellet in 90 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 300 µL of Binding Buffer (L3).
6. Mix well by vortexing.
7. Add 210 µL of 96-100 ethanol.
8. Mix well by vortexing.

Proceed immediately to "Isolate RNA" on page 11.

Prepare lysates from large sample amount

1. Lyse the samples in TRIzol™ Reagent.

Sample type	Lysis procedure
Tissues	Homogenize tissue samples in 1 mL of TRIzol™ Reagent per 50–100 mg of tissue using a tissue homogenizer.
Adherent cells	1. Add 1 mL of TRIzol™ Reagent per 10 cm^2 directly to the culture dish. 2. Lyse the cells by passing the cell lysate several times through a pipette.
Suspension Cells	Harvest cells 1. Harvest and pellet the cells by centrifugation. 2. Add 1 mL of TRIzol™ Reagent per 5–10 × 10^6 animal, plant, or yeast cells, or per 1 × 10^7 bacterial cells. 3. Lyse the cells by passing the cell lysate several times through a pipette.

2. Incubate for 5 minutes.
3. Add 0.2 mL of chloroform per 1 mL of TRIzol™ Reagent, then shake the tube vigorously by hands for 15 seconds.
   Do not vortex the tube.
4. Centrifuge the sample at 12,000 × *g* for 15 minutes at 4°C.
   The mixture separates into a lower red phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. The volume of the aqueous phase is ~600 µL.
5. Transfer ~400 µL of the colorless, upper phase containing RNA to a fresh tube.
6. Add 215 µL of 96–100% ethanol.
7. Mix well by vortexing.

Proceed immediately to "Isolate RNA" on page 11.

Prepare lysates from dicing reaction

Follow this procedure to prepare samples produced in a dicing reaction.

1. Add 300 µL of Binding Buffer (L3) and 300 µL of 96–100% ethanol to 300 µL of the dicing reaction.
2. Mix well by vortexing.

Proceed immediately to "Isolate RNA" on page 11.

Isolate RNA

Bind the RNA to the membrane

1. Transfer sample to a Spin Cartridge with Collection Tube.
2. Centrifuge for 1 minute at 12,000 × *g*.
3. Add 700 µL of 96–100% ethanol.
4. Mix well by vortexing.
5. Transfer 700 µL of the sample to a new Spin Cartridge.
6. Centrifuge for 1 minute at 12,000 × *g*.
7. Transfer remaining sample to the Spin Cartridge from step 5.
8. Discard the flow through, then place the Spin Cartridge into the Collection Tube.

Wash the RNA on the membrane

1. Add 500 µL of Wash Buffer to the Spin Cartridge.
2. Centrifuge for 1 minute at 12,000 × *g*.
3. Repeat step 1-step 2.
4. Discard the flow through, then place the Spin Cartridge into a Wash Tube.
5. Centrifuge for 2–3 minutes at maximum speed.
6. Discard the Wash Tube.

Elute the RNA

1. Place the Spin Cartridge into a clean 1.7–mL Recovery Tube.
2. Add 50–100 µL of Sterile, RNase-free Water to the center of the cartridge.
3. Incubate for 1 minute.
4. Centrifuge for 1 minute at maximum speed.
5. Remove and discard the Spin Cartridge.
   The Recovery Tube contains the purified RNA.

Proceed immediately to downstream application or store the purified RNA at –80°C for further use.

Determine RNA quality and quantity

Estimate RNA quantity

Alternatively, the purified RNA can be quantified using Quant-iT™ RNA Assay Kit (Cat. No. Q33140).

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)

Analysis of the RNA quality

Typically, RNA isolated using the PureLink™ miRNA Isolation 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.

To visualize small RNA molecules, you can perform a denaturing gel electrophoresis using 10% or 15% TBE acrylamide gels (see page 14 for an example).

A Troubleshooting

Observation	Possible cause	Recommended action
The RNA yield is low	The lysis is incomplete or too much sample has clogged the filter.	Decrease the amount of starting material used.
		For tissues, cut the tissue into smaller pieces and ensure that the tissue is completely immersed in Binding Buffer (L3) to obtain optimal lysis.
		If the cartridge is clogged, decrease the sample volume used or load the sample on 2 Spin Cartridges.
	The sample has a low RNA content.	Various tissues have different small RNA content and some tissue may not contain any small RNA at detectable levels.
	The flow through from the first Spin Cartridge was discarded.	Do not discard the flow through from the first cartridge. The flow through contains small RNA molecules.
	The binding conditions were incorrect.	For efficient binding of small RNA molecules, always add ethanol to the flow through to a final concentration of 70%.
	Ethanol was not added to Wash Buffer (W5).	Be sure to add 96–100% ethanol to Wash Buffer (W5) as described in "Before you begin" on page 7.
	The elution conditions were incorrect.	Add Sterile, RNase-free Water to the center of the Spin Cartridge, then incubate for 1 minute before centrifugation.
	The RNA was quantified in water.	Be sure the RNA quantitation using UV absorbance is performed with 10 mM Tris-HCl, pH 7.0 to accurately measure the UV absorbance.
The RNA is degraded	The RNA is contaminated by RNase.	Follow "Procedural guidelines" on page 7.
	The starting materials are of poor quality.	Always use fresh samples or samples frozen at –80°C. For lysis, process the sample quickly to avoid degradation.
The RNA is contaminated by genomic DNA	A large sample amount was loaded onto the first Spin Cartridge.	Follow "Prepare lysates from large sample amount" on page 10.
		Perform DNase I digestion on the RNA sample after elution to remove genomic DNA contamination.
The RNA is contaminated by total RNA	A large sample amount was loaded onto the first Spin Cartridge.	Follow "Prepare lysates from large sample amount" on page 10.

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Expected results

Results

An example of small RNA molecules isolated from various samples is shown in the following figure.

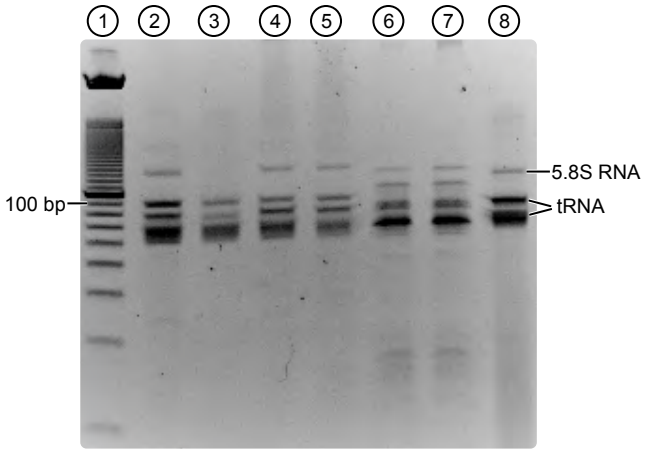

Figure 1 Small RNA molecules from various samples isolated using PureLink™ miRNA Isolation Kit

Samples (10 µL eluate) were analyzed on a Novex TBE-Urea 15% Gel (Cat. No. EC6885BOX) and RNA bands were visualized with ethidium bromide staining after electrophoresis.

① 1 µL 10 bp DNA Ladder
② HeLa cells
③ 293F cells
④ Rat spleen
⑤ Mouse liver
⑥ Spinach
⑦ MaV yeast cells
⑧ *E. coli* cells

Expected yields

The yield of small RNA molecules obtained from various samples using PureLink™ miRNA Isolation Kit is listed in the following table. The RNA quantitation was performed with the Quant-iT™ RNA Assay Kit (Cat. No. Q33140).

Table 2 Yield of total RNA obtained from various sources

Source	Amount	small RNA yield
HeLa cells	1×10^6 cells	1.29 µg
293F cells	1×10^6 cells	1.95 µg
Mouse liver	5 mg	0.71 µg
Rat spleen	5 mg	1.39 µg
Spinach	60 mg	1.64 µg
Yeast (*S. cerevisae*)	1×10^6 cells	6.21 µg
Bacteria (*E. coli*)	2×10^6 cells	0.55 µg

C

One-column protocol

The one-column purification protocol is only applicable to samples obtained from dicing reaction and is designed for use with up to 8 µg of RNA in 50-µL dicing reaction as starting material.

- To process <8 µg in <50-µL dicing reaction, increase the volume of the starting material to 50 µL.
- To process >8 µg in >50-µL dicing reaction, adjust the buffer volumes accordingly and perform multiple loadings of the sample onto the Spin Cartridge.

The one-column protocol is not recommended with samples prepared from tissues or cells as the amount of sample used for purification may clog the Spin Cartridges, and the volume and amount of buffers used for wash and elution steps are not sufficient to produce high-quality RNA.

Bind the RNA to the membrane

1. Add 150 µL of Binding Buffer (L3) to the 50-µL dicing reaction, them mix well.
2. Add 600 µL of 96–100% ethanol, then mix well.
3. Transfer 700 µL of the sample to a Spin Cartridge in a Collection Tube.
4. Centrifuge for 1 minute at 12,000 × *g*, then discard the flow through.

Wash the RNA on the membrane

1. Add 500 µL of Wash Buffer (W5) to the Spin Cartridge.
2. Centrifuge for 1 minute at 12,000 × *g*.
3. Repeat step 1-step 2.
4. Discard the Collection Tube, then place the Spin Cartridge in a Wash Tube.
5. Centrifuge for 1 minute at 12,000 × *g*, then discard the Wash Tube.

Elute the RNA

1. Place the Spin Cartridge in a Recovery Tube, then add 100 µL of Buffer (W4) for single-column purification.
2. Incubate for 1 minute.
3. Centrifuge for 1 minute at 12,000 × *g*, then discard the Spin Cartridge.
   The eluate contains the RNA.

Precipitate the RNA

1. Add 200 µL of cold 96–100% ethanol and 1 µL of glycogen solution (20 µg/µL) to the eluate.
2. Mix well and incubate for 15 minutes at –20°C.
3. Centrifuge for 15 minutes at maximum speed at 4°C.
4. Carefully discard the supernatant without disturbing the pellet.
5. Resuspend the pellet in 0.5 mL of cold 70% ethanol.
6. Centrifuge for 10 minutes at maximum speed at 4°C.
7. Carefully discard the supernatant without disturbing the pellet.
8. Air-dry the pellet for ~5 minutes.
9. Resuspend the pellet in 50 µL of Sterile, RNase-free Water.

Proceed immediately to downstream application or store the purified RNA at –80°C for further use.

D Ordering information

Additional products

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

Item	Quantity	Cat. No.
RNase *AWAY*™ Decontamination Reagent	250 mL	10328011
RNase*Zap*™ RNase Decontamination Solution	250 mL	AM9780
UltraPure™ DEPC-treated Water	1 L	750023
UltraPure™ DNase/RNase-Free Distilled Water	500 mL	10977-015
Quant-iT™ RNA Assay Kit	1 kit	Q33140
Novex TBE-Urea Gels, 15% (1.0 mm, 10 well)	1 box	EC6885BOX
Novex TBE-Urea Gels, 10% (1.0 mm, 10 well)	1 box	EC6875BOX
Novex TBE-Urea Sample Buffer (2X)	10 mL	LC6876
Novex TBE Running Buffer (5X)	1 L	LC6675
Homogenizer	50 each	12183026
10 bp DNA Ladder	50 µg	10821-015
PureLink™ RNA Mini Kit	50 reactions	12183018A
TrackIt™ 10 bp DNA Ladder	20 applications	10488-019
UltraPure™ Glycogen	100 µL	10814-010
TRIzol™ Reagent	100 mL	15596026

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

Documentation and support

Customer and technical support

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- Worldwide contact telephone numbers
- Product support, including:
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- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

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.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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Liu, C. G., Calin, G. A., Meloon, B., Gamliel, N., Sevignani, C., Ferracin, M., Dumitru, C. D., Shimizu, M., Zupo, S., Dono, M., Alder, H., Bullrich, F., Negrini, M., and Croce, C. M. 2004. *An Oligonucleotide Microchip for Genome-wide microRNA Profiling in Human and Mouse Tissues.* Proc. Natl. Acad. Sci. USA 101, 9740-9744.