invitrogen™

PureLink™ RNA Micro Kit

For purification of total RNA from variety of samples in low elution volume

Catalog no. 12183-016

Version B
19 December 2008
100003660

User Manual

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Kit Contents and Storage

Shipping and Storage

All contents of the PureLink™ RNA Micro Kit are shipped at room temperature.

Upon receipt, store the PureLink™ DNase/Carrier RNA box at 4°C. Store the remaining kit components at room temperature.

Kit contents are stable for up to six months when properly stored.

Kit Contents

The components included in the PureLink™ RNA Micro Kit are provided in two boxes, described below. Sufficient reagents are provided to perform 50 preparations.

PureLink™ RNA Micro Kit Contents (Box 1)	**Quantity**
Lysis Buffer	125 ml
Wash Buffer I	50 ml
Wash Buffer II	15 ml
RNase-Free Water	15.5 ml
PureLink™ Micro Kit Columns (with collection tubes)	50 each
Collection Tubes	50 each
Recovery Tubes	50 each

PureLink™ DNase/Carrier RNA (Box 2)	**Quantity**
PureLink™ DNase (lyophilized)	1500 Units
PureLink™ On–Column DNase 2X Buffer	1 ml
RNase-Free Water	1 ml
PureLink™ Carrier RNA (lyophilized)	300 µg

Additional Products

Additional Products

The following products are also available from Invitrogen.

For more details, visit our web site at www.invitrogen.com or contact **Technical Support** (page 43).

RT-PCR and qRT-PCR Products

Product	Quantity	Catalog No.
PureLink™ DNase	50 preps	12185–010
SuperScript® III First-Strand Synthesis System for RT-PCR	50 reactions	18080–051
SuperScript® III First-Strand Synthesis SuperMix	50 reactions	18080–400
SuperScript® III First-Strand Synthesis SuperMix for qRT-PCR	50 reactions 250 reactions	11752–050 11752–250
Platinum® PCR SuperMix	100 reactions	11306–016
Platinum® Quantitative PCR SuperMix-UDG	100 reactions 500 reactions	11730–017 11730–025
SuperScript™ III Platinum® Two-Step qRT-PCR Kit	100 reactions	11734–050

Other Products

Product	Quantity	Catalog No.
Homogenizer	50 pack	12183–026
RNase *AWAY*®	250 ml	10328–011
TRIzol® Reagent	100 ml 200 ml	15596–026 11596–018
TRIzol® LS Reagent	100 ml 200 ml	10296–010 10296–028
TRIzol® Plus RNA Purification System	50 preps	12183–555
0.1–2 Kb RNA Ladder	75 µg	15623–100
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

Introduction

Overview

Introduction

The PureLink™ RNA Micro Kit provides a simple, reliable, and rapid method for isolating high-quality total RNA from a variety of small sample sizes in low elution volumes. The total RNA can be purified from samples including animal cells and tissue and Laser Capture Microdissection samples, and is suitable for use in a variety of downstream applications (see below).

System Overview

This manual provides sample–specific protocols to isolate total RNA from variety of small sample sizes in low elution volumes.

In general, samples are lysed and then homogenized in the presence of guanidinium isothiocyanate, a chaotropic salt capable of protecting the RNA from endogenous RNases (Chirgwin *et al.*, 1979). Ethanol is added after homogenization and the sample is then processed through a PureLink™ Micro Kit Column containing a clear silica-based membrane to which the RNA binds. Any impurities are effectively removed by subsequent washing (Vogelstein & Gillespie, 1979). The purified total RNA is then eluted in RNase-Free Water (Tris Buffer, pH 7.5 may also be used) and is suitable for use in a variety of downstream applications, described below.

Downstream Applications

The purified total RNA eluted using the PureLink™ RNA Micro Kit is suitable for use in a variety of applications, including:

- RNA amplification for microarray analysis
- cDNA library preparation after poly(A)+ selection
- RNA amplification for microarray analysis
- Real-time-PCR (RT-PCR)
- Real-time quantitative–PCR (qRT–PCR)
- Northern blotting
- Nuclease protection assays

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Advantages of the Kit

The PureLink™ RNA Micro Kit offers the following advantages:

- RNA isolation from a variety of sample types with very small quantities of starting material
- Minimal genomic DNA contamination of the purified RNA with on–column DNase digestion
- Rapid and convenient column purification procedures
- Reliable performance of high-quality purified total RNA in downstream applications

Starting Material

The various sample types and amounts that can be processed using the PureLink™ RNA Micro Kit are listed in the table below:

Sample type	Sample Amount	Page
Animal cells	≤5 × 10^5 cells	11
Animal tissue	≤5 mg*	16
Laser Capture Microdissection Samples	–	21

***Note:** Up to 10 mg of animal tissue may be processed if you are using TRIzol® Reagent for lysis.

Kit Specifications

Starting Amount:	Varies with sample type (see table above)
Column Binding Capacity:	>100 µg RNA
Column Reservoir Capacity:	700 µl
Centrifuge Compatibility:	Capable of centrifuging at ≥12,000 × *g*
Elution Volume:	≤12 µl
RNA Yield:	>85%

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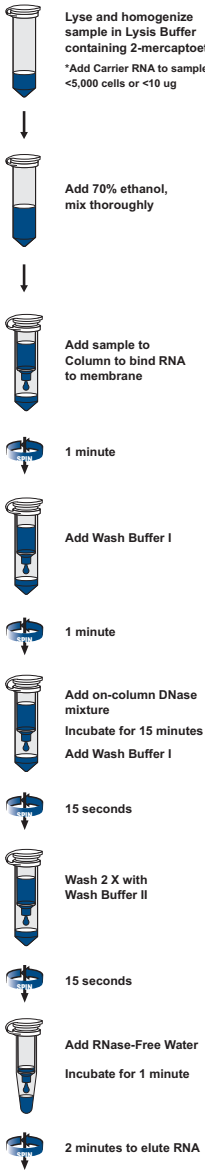
Overview, Continued

Workflow

The flow chart below illustrates the basic steps for isolating total RNA from animal cells and tissue using the PureLink™ RNA Micro Kit.



Methods

General Guidelines

Introduction

Review the information in this section **before** beginning.

Guidelines are provided in this section for handling RNA and sample collection.

Guidelines for Handling RNA

Follow the guidelines below to prevent RNase contamination and to maximize RNA yield.

- Use sterile, disposable, and individually wrapped plastic–ware.
- Use *only* sterile, disposable RNase-free pipet tips and microcentrifuge tubes.
- Wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin. Change gloves frequently, particularly as the protocol progresses from crude extracts to more purified material (*e.g.* from Wash Buffer I to Wash Buffer II).
- Always use proper microbiological aseptic techniques when working with RNA.
- Use RNase *AWAY*® Reagent (page vi) to remove RNase contamination from work surfaces and non-disposable items such as centrifuges and pipets that will be used during purification.

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Guidelines for Sample Collection

When collecting your samples, follow the guidelines below to minimize RNA degradation and to maximize RNA yield.

- Always wear disposable gloves while handling samples and reagents to prevent RNase contamination.
- Work quickly during sample harvesting and use RNase-free dissection tools and containers (scalpels, dishes, tubes etc.).
- Use RNase *AWAY*® Reagent (page vi) to remove RNase contamination from work surfaces.
- When purifying total RNA from fresh samples, keep the fresh cell and tissue samples on ice immediately after harvesting; quickly proceed to sample **Lysis and Homogenization**.
- When purifying total RNA from frozen samples, freeze samples immediately after collection in liquid nitrogen or on dry ice. Keep frozen samples at –80°C or in liquid nitrogen until proceeding to sample **Lysis and Homogenization**.

- Both Lysis Buffer and Wash Buffer I contain guanidine isothiocyanate (an irritant). This chemical is harmful when in contact with the skin, or when it is inhaled or ingested.
- **Do not** add bleach or acidic solutions directly to solutions or sample preparation waste that contains guanidinium hydrochloride, as reactive compounds and toxic gases are formed.
- Ethanol is added to Wash Buffer II. Solutions containing ethanol are considered flammable. Use appropriate precautions when using this chemical.

For your protection, always wear a laboratory coat, gloves and safety glasses when handling these chemicals. Dispose of the buffers and chemicals in appropriate waste containers.

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TRIzol® Reagent

To isolate RNA from samples that are difficult to lyse (*e.g.*, fibrous or fatty animal tissue), or to purify ultrapure total RNA for sensitive downstream applications, you can use TRIzol® Reagent (page vi) followed by purification using the PureLink™ RNA Micro Kit (see page 24 for details).

Microcentrifuge Pestle

RNase-free microcentrifuge pestles allow disruption and lysis of tissue samples in a microcentrifuge tube. They are usually made of Teflon, polyethylene, or stainless steel, and are designed to fit standard microcentrifuge tube sizes (*e.g.* 1.5-ml conical tubes or 2-ml round–bottom tubes).

To use the microcentrifuge pestle:

1. Cool the microcentrifuge tube on ice.
2. Transfer the tissue sample into the microcentrifuge tube.
3. Add Lysis Buffer and use up-and-down with twisting movements to disrupt the sample between the tube wall and the pestle.
4. After lysis, homogenize the sample as specified in your sample-specific protocol.

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Homogenizer

The Homogenizer (page vi) is designed to homogenize cell or tissue lysates via centrifugation, prior to nucleic acid purification. The Homogenizer consists of a cartridge with a specialized membrane that fits inside the Collection Tube that contains the lysate. The Collection Tube is placed into a microcentrifuge, and the Homogenizer homogenizes the lysate by centrifugal force (≥12,000 × *g* for 2 minutes).

The Homogenizer provides highly consistent results and is more convenient than other homogenization methods.

For more details, visit our web site at www.invitrogen.com or contact **Technical Support** (page 43).

Rotor-Stator Homogenizer

Rotor-stator homogenizers allow simultaneous lysis and homogenization of tissue samples or cell lysates by the shearing force of a fast rotating probe.

To use the rotor-stator:

1. Transfer your sample into a 1.5-ml microcentrifuge tube and add the appropriate volume of Lysis Buffer (refer to your sample–specific protocol to determine the amount of Lysis Buffer needed).
2. Insert the rotor-stator probe tip into the sample and homogenize for 5–90 seconds, depending on the toughness of sample.

Note: Avoid foaming of your sample by keeping the tip of the probe submerged in the lysis solution while holding the tip against the tube wall. Refer to the manual provided with your rotor-stator for more information. Rotor-stators are available in various sizes. Common models include ULTRA-TURRAX® (IKA Works, Inc.) and Polytron® Homogenizer (Kinematica, Brinkmann Instruments).

Sample Lysis and Homogenization

Use the tables below and on the next page to determine the best method for lysing and homogenizing your specific sample type.

Sample Type	Lysis Options	Homogenization Options	Comments
Animal Cells	Lysis Buffer, vortexing	• Homogenizer • Syringe and needle • Rotor-stator	
Animal Tissue: Frozen or Fresh Fibrous	Pestle with microcentrifuge tube	• Homogenizer • Syringe and needle	--
	Mortar and pestle in liquid nitrogen	• Homogenizer • Syringe and needle	--
	Rotor-stator		
Animal Tissue: Fresh Soft	Pestle with microcentrifuge tube	• Homogenizer • Syringe and needle	--
	Rotor-stator		Rotor-stator lyses and homogenizes simultaneously and can be used with all tissue amounts.
Laser Capture Microdissection	Lysis Buffer plus vortexing		

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Buffer Preparation and Parameters

Preparing Lysis Buffer with 2-Mercaptoethanol

Prepare a fresh amount of Lysis Buffer containing 1% 2-mercaptoethanol for each purification procedure. Add 10 µl 2-mercaptoethanol for each 1 ml Lysis Buffer.

Refer to your sample–specific protocol for the correct amount of Lysis Buffer with 2-mercaptoethanol to use.

Note

Dithiothreitol (DTT) can be used as an alternative reducing agent in place of 2-mercaptoethanol in the Lysis Buffer.

Prepare a fresh amount of Lysis Buffer containing 40 mM DTT for each purification procedure. Add 20 µl of 2 M DTT for each 1 ml of L3 Lysis Buffer.

Prepare fresh DTT solution, by resuspending 308.5 mg DTT (Invitrogen Cat. No. 15508-013) in 1 ml of RNAse-free water.

Preparing Wash Buffer II with Ethanol

Before using Wash Buffer II for the first time:

1. Add 60 ml of 96–100% ethanol directly to the bottle.
2. Check the box on the Wash Buffer II label to indicate that ethanol was added.
3. Store Wash Buffer II with ethanol at room temperature.

PureLink™ DNase

PureLink™ DNase is optimized for use with the PureLink™ RNA Micro Kit. It is designed to be used specifically for on-column digestion of DNA during critical RNA purification procedures for downstream applications that require DNA–free total RNA.

Resuspending PureLink™ DNase

Resuspend the PureLink™ DNase by dissolving the lyophilized DNase in 550 µl RNase-Free Water (supplied with kit).

Store at 4°C for short-term storage. Thawed DNase stocks may be stored at 4°C for up to three months. For long-term storage, prepare aliquots of the DNase and store at –20°C. Avoid repeat freezing and thawing.

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PureLink™ Carrier RNA

The PureLink™ RNA Micro Kit is supplied with 300 µg of lyophilized PureLink™ Carrier RNA to assist with isolating RNA from samples with low amounts of RNA (≤5,000 cells or ≤10 µg tissue) that would otherwise be lost due to non-specific absorbance.

Preparing PureLink™ Carrier RNA

Prepare a stock solution by resuspending the lyophilized PureLink™ Carrier RNA in 600 µl RNase-Free Water for a final concentration of 500 ng/µl.

Store Carrier RNA stocks at 4°C for up to three months. For long-term storage, prepare aliquots of the Carrier RNA and store at –20°C. Avoid repeat freezing and thawing.

To use the PureLink™ Carrier RNA during lysis, prepare a **1:100 dilution of Carrier RNA** as follows:

For normal Lysis:

1. Add 5 µl Carrier RNA stock (see above) to 495 µl of L3 Lysis Buffer for a final concentration of 5 ng/µl.
2. Add 5 µl diluted Carrier RNA from Step 1 per 350 µl prepared RNA lysis solution (or per volume of lysis) used for a single reaction (25 ng/350 µl).

For lysis with TRIzol®:

1. Add 5 µl Carrier RNA stock (see above) to 495 µl RNase-free water for a final concentration of 5 ng/µl.
2. Add 5 µl diluted Carrier RNA from Step 1 per 350 µl prepared RNA lysis solution (or per volume of lysis) used for a single reaction (25 ng/350 µl).

Elution Parameters

Elution Reagent

RNA can be eluted from the PureLink™ Micro Kit Column using RNase-Free Water (included in the kit). Alternatively, you may use Tris Buffer (10 mM Tris-HCl), pH 7.5 in RNase-free water to elute your RNA.

Elution Volume

RNA yield is dependent on sample type, size, and quality. Depending on your expected RNA yield, and your sample source and starting amount, use between 12 µl–22 µl RNase Free Water (or Tris-Buffer) for each elution. Example yields for various sample types and amounts are provided on page 39. **The dead volume of the cartridge is ~2 µl**. An elution volume of 12 µl will result in a final eluate volume of 10 µl.

Purifying RNA from Animal Cells

Introduction

This section provides instructions for purifying total RNA from ≤5 × 10^5animal cells. Separate protocols are provided for cells in suspension and monolayer.

Materials Needed

You will need the following items in addition to the kit components:

- 2-mercaptoethanol
- 70% ethanol (in RNase-Free Water)
- Microcentrifuge capable of centrifuging ≥12,000 × *g*
- 1.5 ml RNase-free microcentrifuge tubes
- RNase-free pipet tips
- PureLink™ DNase (prepared as described on page 9)
- PureLink™ Carrier RNA (optional, if using <5,000 cells)
- **One** of the following for homogenization:

  Homogenizer (see page vi and page 7) **or,**

  RNase-free syringe (1 ml) with 18–21-gauge needle **or,**

  Rotor-stator homogenizer (page 7)

Note

For samples that are difficult to lyse, you can use TRIzol® Reagent followed by purification using the PureLink™ RNA Micro Kit, (see page 24).

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Lysis and Homogenization ≤5 × 10^5 Suspension Cells

Before beginning, prepare all buffers and solutions according to protocol on pages 9–10.

Follow the steps below to prepare lysates from ≤5 × 10^5 suspension cells:

1. Transfer cells to an appropriately sized RNase-free tube and centrifuge at 2,000 × *g* for 5 minutes at 4°C to pellet. Discard the growth medium from the tube.
2. Add the 350 µl Lysis Buffer prepared with 2-mercaptoethanol (page 9) to your sample.

   Note: If you are processing fewer than 5,000 cells, add 5 µl of diluted PureLink™ Carrier RNA (25 ng/350 µl, prepared as described on page 10).
3. Vortex at high speed until the cell pellet is completely dispersed and the cells appear lysed.

   Note: If you are using a rotor-stator, you may skip this step.
4. Proceed with **one** of the following homogenization options at room temperature:
   - Transfer the lysate to a Homogenizer (page vi) inserted in a Collection Tube and centrifuge at ≥12,000 × *g* for 2 minutes. Remove the Homogenizer when done, **or**
   - Pass the lysate 5–10 times through an 18–21-gauge needle attached to an RNase-free syringe, **or**
   - Transfer the lysate to an appropriately sized RNase-free tube and homogenize using a rotor-stator homogenizer at maximum speed for at least 45 seconds.

Proceed to **Binding, Washing, and Elution**, page 14.

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Lysis and Homogenization ≤5 × 10^5 Monolayer Cells

Before beginning, prepare all buffers and solutions according to protocol on pages 9–10.

Follow the steps below to prepare lysates from ≤5 × 10^5 monolayer cells:

1. Remove the growth medium from the cells.
2. Add 350 µl Lysis Buffer prepared with 2-mercaptoethanol (page 9) evenly over your monolayer.

   Note: If you are processing fewer than 5,000 cells, add 5 µl of diluted PureLink™ Carrier RNA (25 ng/350 µl, prepared as described on page 10).
3. Pipet the cells up and down until they appear lysed.
4. Proceed with **one** of the following homogenization options at room temperature:

   - Transfer the lysate to a Homogenizer (page vi) inserted in a Collection Tube and centrifuge at ≥12,000 × *g* for 2 minutes. Remove the Homogenizer when done, **or**
   - Transfer the lysate to a 1.5 ml RNase-free tube and pass 5–10 times through an 18–21-gauge needle attached to an RNase-free syringe, **or**
   - Transfer the lysate to an appropriately sized RNase-free tube and homogenize using a rotor-stator homogenizer at maximum speed for at least 45 seconds.

Proceed to **Binding, Washing, and Elution**, next page.

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Binding, Washing, and Elution

Before beginning, prepare all buffers and solutions according to protocol on pages 9–10.

Follow the steps below to bind, wash, and elute the RNA from your cell sample:

1. Add 350 µl (or equivalent volume) 70% ethanol to each volume of prepared cell homogenate.

   Note: If part of the sample was lost during homogenization, adjust the volume of ethanol accordingly.

2. Vortex to mix thoroughly and to disperse any visible precipitate that may form after adding ethanol.
3. Transfer up to 700 µl of the sample (including any remaining precipitate) to PureLink™ Micro Kit Column (with the Collection Tube).
4. Centrifuge at ≥12,000 × *g* for 1 minute at room temperature. Discard the flow-through, and reinsert the Column into the same Collection Tube.
5. **Repeat** Steps 3–4 until the entire sample has been processed.
6. Add 350 µl Wash Buffer I to the Column.

   Note: If On-Column DNase is not being performed, increase the volume of Wash Buffer I to 600 µl. Centrifuge at ≥12,000 × g for 15 seconds at room temperature. Discard the flow-through and place the Column into a **new** Collection Tube and proceed to Step 13.

7. Centrifuge at ≥12,000 × g for 1 minute at room temperature. Discard the flow-through **and** the Collection Tube. Insert the Column into a **new** Collection Tube.
8. Add 10 µl Reconstituted PureLink™ DNase (page 9) to 10 µl 2X DNase Buffer for a total of 20 µl. Mix by gently pipetting up and down several times.
9. Pipet all 20 µl of the DNase mixture onto the center of the Column membrane.
10. Incubate at room temperature for 15 minutes.

Procedure continued on next page

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Binding, Washing, and Elution, continued

Procedure continued from previous page

11. Add 350 µl Wash Buffer I to the Column.
12. Centrifuge at ≥12,000 × *g* for 15 seconds at room temperature. Discard the flow-through.
13. Add 500 µl Wash Buffer II with ethanol (page 9) to the PureLink™ Micro Kit Column.
14. Centrifuge at ≥12,000 × *g* for 15 seconds at room temperature. Discard the flow-through.
15. Repeat Steps 13–14 **once**.
16. Centrifuge the Column at ≥12,000 × *g* for 1 minute to dry the membrane with attached the RNA. **Discard** the flow-through and the Collection Tube and insert the PureLink™ Micro Kit Column into a Recovery Tube.
17. Add 12–22 µl RNase-Free Water to the center of the PureLink™ Micro Kit Column (see **Elution Parameters**, page 10).
18. Incubate at room temperature for 1 minute.
19. Centrifuge the Column for 2 minutes at ≥12,000 × *g* at room temperature to elute the RNA from the membrane into the Recovery tube.

    Note: The dead volume of the PureLink™ Micro Kit Column is ~2 µl. An elution volume of 12 µl will result in a final elute volume of 10 µl.
20. Store your purified RNA or proceed to **Analyzing RNA Yield and Quality** (page 37).

Storage of Purified RNA

Store your purified RNA on ice if you will use the RNA within a few hours. For long-term storage, store your purified RNA at –80°C.

Purifying RNA from Animal Tissues

Introduction

This section provides protocols for purifying total RNA from ≤5 mg of fresh or frozen animal tissue.

Note

For samples that are difficult to lyse, or to purify ultrapure total RNA for downstream applications, you can use TRIzol® Reagent followed by purification using the PureLink™ RNA Micro Kit, (page 24).

Important

- Frozen tissue must remain frozen at –80°C prior to lysis. Cool RNase-free tubes on dry ice before placing the frozen tissue in them. **Thawing of frozen tissue prior to lysis may result in RNA degradation and reduced RNA yield.**
- Fast and complete disruption of tissue during lysis is important to prevent RNA degradation.

Summary of Lysis Methods

The following table provides a summary of lysis methods based on sample type and size.

Tissue type	Available lysis methods
Frozen or Fresh fibrous	• Microcentrifuge Pestle • Rotor-stator
Fresh soft	• Microcentrifuge Pestle • Rotor-stator

Continued on next page

Materials Needed

You will need the following items in addition to the kit components:

- 2-mercaptoethanol
- 70% ethanol (in RNase-Free Water)
- 96–100% ethanol
- PureLink™ DNase (prepared as described on page 9)
- PureLink™ Carrier RNA (optional if ≤10 µg tissue)
- 1.5-ml RNase-free tubes
- Microcentrifuge capable of centrifuging ≥12,000 × *g*
- RNase-free pipet tips
- RNase-free glass, Teflon, **or** plastic pestle
- **One** of the following for homogenization:
  18–20-gauge needle with RNase-free syringe **or**
  Homogenizer (page vi and page 7) **or**
  Rotor-stator homogenizer (page 7)

Note

For samples that are difficult to lyse, you can use TRIzol® Reagent followed by purification using the PureLink™ RNA Micro Kit, (see page 24).

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Lysis and Homogenization ≤5 mg Frozen or Fresh Tissue

Before beginning, prepare all buffers and solutions according to protocol on pages 9–10.

Use **one** of the following protocols (microcentrifuge pestle, or rotor-stator) to prepare frozen or fresh fibrous tissue.

Microcentrifuge Pestle Protocol

1. Transfer up to 5 mg tissue sample to appropriately sized microcentrifuge tube.
2. Immediately add 350 µl Lysis Buffer prepared with 2-mercaptoethanol (page 9) to your sample.
   Note: If you are processing ≤10 µg tissue, add 5 µl of diluted PureLink™ Carrier RNA (25 ng/350 µl, prepared as described on page 10).
3. Mince the tissue using an appropriately sized RNase-free pestle. Use up/down and twisting movements in the tube until tissue is thoroughly disrupted and lysed.
4. Centrifuge at ≥12,000 × g for 2 minutes at room temperature. Transfer the supernatant to a new RNase-free microcentrifuge tube.
5. Proceed with **one** of the following homogenization options at room temperature:
   - Transfer the lysate to a Homogenizer (page vi) inserted in an RNase-ree tube and centrifuge at ≥12,000 × *g* for 2 minutes. Remove the Homogenizer when done, **or**
   - Pass the lysate 5–10 times through an 18–21-gauge needle attached to an RNase-free syringe, then centrifuge at ≥12,000 × *g* for 2 minutes. Transfer the supernatant to a new RNase-free tube.

Proceed to **Binding, Washing, and Elution** (next page).

Rotor-Stator Protocol:

1. Transfer your tissue sample to an appropriately sized microcentrifuge tube.
2. Immediately add 350 µl Lysis Buffer prepared with 2-mercaptoethanol (see page 9) to your sample using RNase-free pipet tips.
3. Quickly homogenize your sample using the rotor–stator at maximum speed for at least 45 seconds.
4. Centrifuge at ≥12,000 × g for 2 minutes at room temperature.
5. Carefully transfer the supernatant to a new tube.
   Note: Failure to perform Steps 4–5 will lead to column clogging

Proceed to **Binding, Washing, and Elution** (next page).

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Binding, Washing, and Elution

Follow the steps below to bind, wash, and elute the RNA from your tissue sample:

1. Add 350 µl (or equivalent volume) 70% ethanol to each volume of tissue homogenate.

   Note: If part of the sample was lost during homogenization, adjust the volume of ethanol accordingly.

2. Mix thoroughly by shaking or vortexing to disperse any visible precipitate that may form after adding ethanol.
3. Transfer up to 700 µl of the sample (including any remaining precipitate) to the PureLink™ Micro Kit Column (with the Collection Tube).
4. Centrifuge at ≥12,000 × *g* for 1 minute at room temperature. Discard the flow-through, and reinsert the PureLink™ Micro Kit Column in the same Collection Tube.
5. **Repeat** Steps 3–4 until the entire sample has been processed.
6. Add 350 µl Wash Buffer I to the spin cartridge.

   Note: If On column DNase step is not being performed, increase the volume of Wash Buffer I to 600ul. Centrifuge at ≥12,000 × *g* for 30 seconds at room temperature. Discard the flow-through and the collection tube and proceed to step 13.

7. Centrifuge at ≥12,000 × *g* for 1 minute at room temperature. **Discard** the flow-through and the collection tube and place the Column into a **new** Collection Tube.
8. Add 10 µl reconstituted PureLink™ DNase to 10 µl 2X DNase Buffer to obtain a 20 µl mixture. Gently mix by pipetting up and down several times.
9. Add the 20 µl DNase mixture to the center of the Column.
10. Incubate at room temperature for 15 minutes.
11. Add 350 µl Wash Buffer I to the center of the Column.
12. Centrifuge for ≥12,000 × g for 15 seconds at room temperature.

Procedure continued on next page

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Binding, Washing, and Elution, continued

Procedure continued from previous page

13. Add 500 µl Wash Buffer II, prepared with ethanol (page 9) to Column.
14. Centrifuge at ≥12,000 × *g* for 15 seconds at room temperature. Discard the flow-through.
15. Repeat Steps 13–14, **once**.
16. Centrifuge the PureLink™ Micro Kit Column at ≥12,000 × *g* for 1 minute at room temperature to dry the membrane with attached RNA. **Discard** the Collection Tube and insert the PureLink™ Micro Kit Column into a Recovery Tube.
17. Add 12–22 µl RNase-Free Water to the center of the PureLink™ Micro Kit Column (see **Elution Parameters**, page 10).
18. Incubate at room temperature for 1 minute.
19. Centrifuge for 1 minute at ≥12,000 × *g* at room temperature.

    Note: The dead volume of the PureLink™ Micro Kit Column is ~2 µl. An elution volume of 12 µl will result in a final elute volume of 10 µl (see **Elution Parameters,** page 10).
20. Store your purified RNA or proceed to **Analyzing RNA Yield and Quality** (page 37).

Storage of Purified RNA

Store your purified RNA on ice if you will use the RNA within a few hours. For long-term storage, store your purified RNA at –80°C.

Purifying RNA from Laser Capture Microdissection Samples

Introduction

This section provides a protocol for purifying total RNA from Laser Capture Microdissection (LCM) samples.

Materials Needed

You will need the following items in addition to the kit components:

- 2-mercaptoethanol
- 96–100% ethanol
- RNase-free pipet tips
- 1.5–ml RNase-free microcentrifuge tubes
- Microcentrifuge capable of centrifuging ≥12,000 × *g*
- PureLink™ DNase (prepared as described on page 9))
- PureLink™ Carrier RNA (prepared as described on page 10)

Lysis and Homogenization of LCM Samples

Before beginning, prepare all buffers and solutions according to protocol on pages 9–10.

Use the following protocol to prepare your LCM samples.

1. Add 350 µl Lysis Buffer prepared with 2-mercaptoethanol.
2. Add 5 µl diluted PureLink™ Carrier RNA (25 ng/350 µl, prepared as described on page 10).
3. Vortex to lyse and homogenize your sample.
4. Adjust the volume in the tube to 350 µl by adding additional Lysis Buffer, if needed.

   Important: If your sample tube cannot hold 350 ul, transfer the homogenized lysate to a new 1.5 ml RNase-free microcentrifuge tube, and adjust the sample volume to 350 ul by adding Lysis Buffer.

Proceed to **Binding, Washing, and Elution** (below).

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Purifying RNA from Laser Capture Microdissection Samples, Continued

Binding, Washing, and Elution

Follow the steps below to bind, wash, and elute your RNA.

1. Add 350 µl (or equivalent volume) 70% ethanol to each volume of sample homogenate.
2. Mix thoroughly by vortexing to disperse any visible precipitate that may form after adding ethanol.
3. Transfer up to 700 µl of your sample (including any remaining precipitate) to the PureLink™ Micro Kit Column (with a Collection Tube).
4. Centrifuge at ≥12,000 × *g* for 1 minute at room temperature. Discard the flow-through, and reinsert the PureLink™ Micro Kit Column in the same Collection Tube.
5. **Repeat** Steps 3–4 until the entire sample has been processed.
6. Add 350 µl Wash Buffer I to the spin cartridge.

   Note: If On column DNase step is not being performed, increase the volume of Wash Buffer I to 600ul. Centrifuge at ≥12,000 × *g* for 30 seconds at room temperature. Discard the flow-through and the collection tube and proceed to step 13.
7. Centrifuge at ≥12,000 × *g* for 1 minute at room temperature. Discard the flow-through and the collection tube. Insert the Column into a **new** Collection Tube.
8. Add 10 µl reconstituted PureLink™ DNase to 10 µl 2X DNase Buffer to obtain a 20 µl mixture. Gently mix by pipetting up and down several times.
9. Add the 20 µl DNase mixture to the center of the Column.
10. Incubate at room temperature for 15 minutes.
11. Add 350 µl Wash Buffer I to the center of the Column.
12. Centrifuge for ≥12,000 × g for 15 seconds at room temperature.

Procedure continued on next page

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Purifying RNA from Laser Capture Microdissection Samples, Continued

Binding, Washing, and Elution, continued

Procedure continued from previous page

13. Add 500 µl Wash Buffer II with ethanol (page 9) to the PureLink™ Micro Kit Column.
14. Centrifuge at ≥12,000 × *g* for 15 seconds at room temperature. Discard the flow-through and reinsert the PureLink™ Micro Kit Column in the same Collection Tube.
15. Repeat Steps 13–14 **once**.
16. Centrifuge the Column with Collection tube at ≥12,000 × *g* for 1 minute at room temperature to dry the membrane with attached RNA. Discard the Collection Tube and insert the PureLink™ Micro Kit Column into a Recovery Tube.
17. Add 12–22 µl RNase-Free Water to the center of the PureLink™ Micro Kit Column, (see **Elution Parameters,** page 10).
18. Incubate at room temperature for 1 minute.
19. Centrifuge the PureLink™ Micro Kit Column for 1 minute at ≥12,000 × *g* at room temperature.

    Note: The dead volume of the PureLink™ Micro Kit Column is ~2 µl. An elution volume of 12 µl will result in a final elute volume of 10 µl (see **Elution Parameters,** page 10).
20. Store your purified RNA or proceed to **Analyzing RNA Yield and Quality** (page 37).

Storage of Purified RNA

Store your purified RNA on ice if you will use the RNA within a few hours. For long-term storage, store your purified RNA at –80°C.

Using TRIzol® Reagent with the PureLink™ RNA Micro Kit

Introduction

This section provides instructions for using TRIzol® Reagent (page vi) in conjunction with the PureLink™ RNA Micro Kit to isolate total RNA from samples that are difficult to lyse (*e.g.,* fibrous tissues). This combined protocol also allows you to purify ultrapure total RNA for sensitive downstream applications such as qPCR or microarray analysis.

To obtain high-quality total RNA, be sure to follow the **Guidelines for Handling RNA** (page 4).

Materials Needed

You will need the following items:

- TRIzol® Reagent (page vi)
- Chloroform
- PureLink™ DNase (prepared as described on page 9)
- PureLink™ Carrier RNA (optional for ≤10 µg tissue)
- 96–100% ethanol or 70% ethanol (in RNase-free water), depending on protocol option used
- Microcentrifuge capable of centrifuging ≥12,000 × *g*
- 1.5 ml RNase-free microcentrifuge tubes
- RNase-free pipet tips
- Rotor-stator homogenizer **or** Tissue homogenizer

Continued on next page

Using TRIzol® Reagent with the PureLink™ RNA Micro Kit, Continued

TRIzol® Reagent contains phenol (toxic and corrosive) and guanidine isothiocyanate (an irritant), and may be a health hazard if not handled properly. Avoid direct contact with TRIzol® Reagent, as direct contact of skin, eyes, or respiratory tract with TRIzol® Reagent may cause chemical burns to the exposed area.

When working with TRIzol® Reagent, **always** work in a fume hood, and always wear a lab coat, gloves and safety glasses. Refer to the TRIzol® Reagent product insert for more details.

Contact your Environmental Heath and Safety (EH&S) department for proper work and disposal guidelines.

Lysate Preparation with TRIzol® Reagent

Before beginning, prepare all buffers and solutions according to protocol on pages 9–10.

Use TRIzol® Reagent to prepare lysates from various sample types as described below. Refer to the TRIzol® Reagent manual for more information.

***Important:** If you are processing ≤5,000 cells or ≤10 µg tissue, add 5 µl diluted PureLink™ Carrier RNA (25 ng/350 µl, prepared as described on page 10) to your sample after the addition of TRIzol® and before homogenization.

Tissues

Homogenize up to 10 mg of tissue in 1 ml TRIzol® Reagent using a rotor–stator homogenizer.*

Adherent Cells

Lyse cells directly in a culture dish by adding 1 ml of TRIzol® Reagent to the dish and passing the cell lysate several times through an RNase-free pipet tip. The amount of TRIzol® Reagent required is based on the culture dish area (1 ml per 10 cm^2) and not on the number of cells present.*

Suspension Cells

Harvest cells and pellet by centrifugation. Use 1 ml of TRIzol® Reagent per 1×10^6 animal cells. Lyse cells by repetitive pipetting up and down.*

Continued on next page

Phase Separation with TRIzol® Reagent

Following cell or tissue lysis as described above, perform the following steps to isolate the sample.

1. Incubate the lysate with TRIzol® (previous page) at room temperature for 5 minutes to allow complete dissociation of nucleoprotein complexes.
2. Add 0.2 ml chloroform per 1 ml TRIzol® Reagent used. Shake the tube vigorously by hand for 15 seconds.

   Note: Vortexing may increase DNA contamination of your RNA sample. Avoid vortexing if your downstream application is sensitive to the presence of DNA.
3. Incubate at room temperature for 2–3 minutes.
4. Centrifuge the sample at ≥12,000 × *g* for 15 minutes at 4ºC.

   Note: After centrifugation, the mixture separates into a lower, red phenol-chloroform phase, an interphase, and a colorless upper aqueous phase which contains the RNA. The volume of the aqueous upper phase is ~600 µl.
5. Transfer the colorless, upper phase containing the RNA to a new RNase-free tube.
6. Add an equal volume 70% ethanol to obtain a final ethanol concentration of 35%. Vortex to mix well.
7. Invert the tube to disperse any visible precipitate that may form after adding ethanol.

Proceed to **Binding, Washing, and Elution**, next page.

Continued on next page

Binding, Washing, and Elution

Follow the steps below to bind, wash, and elute RNA from your sample.

1. Transfer up to 700 µl of sample (prepared as described on the previous page) to a PureLink™ Micro Kit Column (with a Collection Tube).
2. Centrifuge at ≥12,000 × *g* for 1 minute at room temperature. Discard the flow-through.
3. Add 350 µl Wash Buffer I to the spin cartridge.
   Note: If On column DNase step is not being performed, increase the volume of Wash Buffer I to 600ul. Centrifuge at ≥12,000 × *g* for 30 seconds at room temperature. Discard the flow-through and the collection tube and proceed to step 10.
4. Centrifuge at ≥12,000 × *g* for 1 minute at room temperature. Discard the flow-through **and** the Collection Tube. Insert the Column into a **new** Collection Tube.
5. Add 10 µl reconstituted PureLink™ DNase to 10 µl 2X DNase Buffer to obtain a 20 µl mixture. Gently mix by pipetting up and down several times.
6. Add the 20 µl DNase mixture to the center of the Column.
7. Incubate at room temperature for 15 minutes.
8. Add 350 µl Wash Buffer I to the center of the Column.
9. Centrifuge for ≥12,000 × g for 15 seconds at room temperature.
10. Add 500 µl Wash Buffer II with ethanol (page 9) to the Column.
11. Centrifuge at ≥12,000 × *g* for 15 seconds at room temperature. Discard the flow-through.
12. Repeat Steps 10–11 **once**.

Procedure continued on next page

Continued on next page

Binding, Washing, and Elution

Procedure continued from previous page

13. Centrifuge the Column with Collection tube at ≥12,000 × *g* for 1 minute at room temperature to dry the membrane with attached RNA. Discard the flow-through **and** the Collection Tube and insert the PureLink™ Micro Kit Column into a Recovery Tube.
14. Add 12–22 µl RNase-Free Water to the center of the PureLink™ Micro Kit Column, (see **Elution Parameters,** page 10).
15. Incubate at room temperature for 1 minute.
16. Centrifuge the PureLink™ Micro Kit Column for 1 minute at ≥12,000 × *g* at room temperature.

    Note: The dead volume of the PureLink™ Micro Kit Column is ~2 µl. An elution volume of 12 µl will result in a final elute volume of 10 µl (see **Elution Parameters,** page 10).
17. Store your purified RNA or proceed to **Analyzing RNA Yield and Quality** (page 37).

Storage of Purified RNA

Store your purified RNA on ice if you will use the RNA within a few hours. For long-term storage, store your purified RNA at –80°C.

TRIzol® Plus Total Transcriptome Isolation

Introduction

This section provides instructions for using TRIzol® Reagent (page vi) in conjunction with the PureLink™ RNA Micro Kit to isolate total transcriptome RNA, total RNA, including small RNA's such as miRNA from ≤10 mg of fresh or frozen tissue. This combined protocol also allows you to purify ultrapure total RNA for sensitive downstream applications such as qPCR or microarray analysis.

To obtain high-quality total RNA, be sure to follow the **Guidelines for Handling RNA** (page 4).

Materials Needed

You will need the following items:

- TRIzol® Reagent (page vi)
- Chloroform
- 100% ethanol
- PureLink™ Carrier RNA (optional, if you are using ≤5,000 cells or ≤10 µg tissue)
- Microcentrifuge capable of centrifuging ≥12,000 × *g*
- 1.5 ml RNase-free microcentrifuge tubes
- RNase-free pipet tips
- Rotor-stator homogenizer **or** Tissue homogenizer

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TRIzol® Reagent contains phenol (toxic and corrosive) and guanidine isothiocyanate (an irritant), and may be a health hazard if not handled properly. Avoid direct contact with TRIzol® Reagent, as direct contact of skin, eyes, or respiratory tract with TRIzol® Reagent may cause chemical burns to the exposed area.

When working with TRIzol® Reagent, **always** work in a fume hood, and always wear a lab coat, gloves and safety glasses. Refer to the TRIzol® Reagent product insert for more details.

Contact your Environmental Heath and Safety (EH&S) department for proper work and disposal guidelines.

Lysate Preparation with TRIzol® Reagent

Before beginning, prepare all buffers and solutions according to protocol on pages 9–10.

Use TRIzol® Reagent to prepare lysates from various sample types as described below. Refer to the TRIzol® Reagent manual for more information.

***Important:** If you are processing ≤5,000 cells or ≤10 µg tissue, add 5 µl diluted PureLink™ Carrier RNA (25 ng/350 µl, prepared as described on page 10) to your sample after the addition of TRIzol® and before homogenization.

Tissues

Homogenize up to 10 mg of tissue in 1 ml TRIzol® Reagent using a rotor–stator homogenizer.*

Adherent Cells

Lyse cells directly in a culture dish by adding 1 ml of TRIzol® Reagent to the dish and passing the cell lysate several times through an RNase-free pipet tip. The amount of TRIzol® Reagent required is based on the culture dish area (1 ml per 10 cm^2) and not on the number of cells present.*

Suspension Cells

Harvest cells and pellet by centrifugation. Use 1 ml of TRIzol® Reagent per 1×10^6 animal cells. Lyse cells by repetitive pipetting up and down.*

Continued on next page

TRIzol® Plus Total Transcriptome Isolation, Continued

Phase Separation with TRIzol® Reagent

Following cell or tissue lysis as described above, perform the following steps to isolate the sample.

1. Incubate the lysate with TRIzol® (previous page) at room temperature for 5 minutes to allow complete dissociation of nucleoprotein complexes.
2. Add 0.2 ml chloroform per 1 ml TRIzol® Reagent used. Cap and shake the tube vigorously by hand for 15 seconds.

   Note: Vortexing may increase DNA contamination of your RNA sample. Avoid vortexing if your downstream application is sensitive to the presence of DNA.
3. Incubate at room temperature for 2–3 minutes.
4. Centrifuge the sample at ≥12,000 × *g* for 15 minutes at 4ºC.

   Note: After centrifugation, the mixture separates into a lower, red phenol-chloroform phase, an interphase, and a colorless upper aqueous phase which contains the RNA. The volume of the aqueous upper phase is ~600 µl.
5. Transfer the colorless, upper phase containing the RNA to a new RNase-free tube.
6. Add an equal volume 100% ethanol to obtain a final ethanol concentration of 50%. Vortex to mix well.
7. Invert the tube to disperse any visible precipitate that may form after adding ethanol.

Proceed to **Binding, Washing, and Elution**, next page.

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Binding, Washing, and Elution

Follow the steps below to bind, wash, and elute RNA from your sample.

Note: The total Transcriptome protocol does not contain a Wash Buffer I step.

1. Transfer up to 700 µl of sample (prepared as described on the previous page) to a PureLink™ Micro Kit Column (with a Collection Tube).
2. Centrifuge at ≥12,000 × *g* for 1 minute at room temperature. **Discard** the flow-through.
3. Transfer any remaining sample to the Column and **repeat** Step 2, **once**, discard the flow-through **and** the collection tube and insert the Column into a **new** Collection Tube.
4. Add 500 µl Wash Buffer II with ethanol (page 9) to the center of the PureLink™ Micro Kit Column.
5. Centrifuge at ≥12,000 × *g* for 15 seconds at room temperature. **Discard** the flow-through.
6. Repeat Steps 4–5 **once.**
7. Centrifuge at ≥12,000 × *g* for 1 minute at room temperature to dry the membrane with attached RNA. Discard the flow-through **and** the Collection Tube and insert the PureLink™ Micro Kit Column into a Recovery Tube.

Procedure continued on next page

Continued on next page

Binding, Washing, and Elution

Procedure continued from previous page

8. Add 12–22 µl RNase-Free Water to the center of the PureLink™ Micro Kit Column (see **Elution Parameters**, page 10).
9. Incubate at room temperature for 1 minute.
10. Centrifuge the PureLink™ Micro Kit Column with the Recovery Tube for 2 minutes at ≥12,000 × *g* at room temperature.

    Note: The dead volume of the PureLink™ Micro Kit Column is ~2 µl. An elution volume of 12 µl will result in a final elute volume of 10 µl (see **Elution Parameters,** page 10).
11. Store your purified RNA or proceed to **Analyzing RNA Yield and Quality** (page 37).

Storage of Purified RNA

Store your purified RNA on ice if you will use the RNA within a few hours. For long-term storage, store your purified RNA at –80°C.

Purifying Liquid Samples for RNA Clean–Up and Concentration

Introduction

This section provides instructions for purifying ≥50 µg of liquid sample for RNA clean–up of (*e.g.* enzymatic reactions) or for concentrating ≥50 µg of dilute RNA samples.

Liquid Sample Types

The types of liquid sample supported by this kit include enzymatic reactions (DNase digestion, RNA labeling), cytoplasmic RNA extracts from mammalian cells, and *in vitro* transcription reactions (Sambrook *et al.*, 1989). This kit can also be used to clean up liquid RNA samples.

Materials Needed

You will need the following items in addition to the kit components:

- 2-mercaptoethanol
- 96–100% ethanol
- Wash Buffer II (prepared with ethanol, page 9)
- RNase-Free Water (supplied with kit)
- Microcentrifuge capable of centrifuging ≥12,000 × *g*
- 1.5 ml RNase-free microcentrifuge tubes
- RNase-free pipet tips

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Purifying Liquid Samples for RNA Clean–Up and Concentration, Continued

Purifying RNA from Liquid Samples

Use the following protocol to purify total RNA from liquid samples:

1. To one volume of liquid sample (≤1.2 ml), add one volume Lysis Buffer prepared with 2-mercaptoethanol (page 9) followed by the same volume of 96–100% ethanol (*e.g.,* to 1 ml of sample, add 1 ml of Lysis Buffer followed by 1 ml of ethanol).
2. Mix by vortexing or pipetting up and down 5 times (use RNase-free pipet tips).
3. Transfer up to 700 µl of sample to a PureLink™ Micro Kit Column (with a Collection Tube).
4. Centrifuge at ≥12,000 × *g* for 15 seconds at room temperature. **Discard** the flow-through.
5. **Repeat** Steps 3–4 until the entire sample is processed.
6. Add 500 µl Wash Buffer II with ethanol (page 9) to the PureLink™ Micro Kit Column.
7. Centrifuge at ≥12,000 × *g* for 15 seconds at room temperature. **Discard** flow-through.
8. Repeat Steps 6–7 **once.**

Procedure continued on next page

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Purifying Liquid Samples for RNA Clean–Up and Concentration, Continued

Purifying RNA from Liquid Samples, continued

Procedure continued from previous page

9. Centrifuge the Column at ≥12,000 × *g* for 1 minute at room temperature to dry the membrane with attached RNA. Discard the flow-through **and** the Collection Tube and insert the PureLink™ Micro Kit Column into a Recovery Tube.
10. Add 12–22µl RNase-Free Water to the center of the PureLink™ Micro Kit Column (see **Elution Parameters**, page 10).
11. Incubate at room temperature for 1 minute.
12. Centrifuge the Column for 1 minute at ≥12,000 × *g* at room temperature.

    Note: The dead volume of the PureLink™ Micro Kit Column is ~2 µl. An elution volume of 12 µl will result in a final elute volume of 10 µl (see **Elution Parameters,** page 10).
13. Store your purified RNA or proceed to **Analyzing RNA Yield and Quality** (page 37).

Storage of Purified RNA

Store your purified RNA on ice if you will use the RNA within a few hours. For long-term storage, store your purified RNA at –80°C.

Analyzing RNA Yield and Quality

Introduction

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

RNA Yield

Total RNA is easily quantitated using the Quant-iT™ RiboGreen® RNA Assay Kit or UV absorbance at 260 nm.

Quant-iT™ RiboGreen® RNA Assay Kit

The Quant-iT™ RNA Assay Kit (page vi) provides a rapid, sensitive, and specific method for RNA quantitation with minimal interference from DNA, protein, or other common contaminants that affect UV absorbance readings.

The kit contains a state-of-the-art quantitation reagent and pre-diluted standards for standard curve. The assay is performed in a microtiter plate format and is designed for reading in standard fluorescent microplate readers.

UV Absorbance

To determine the quantity by UV absorbance:

1. Dilute an aliquot of the total RNA sample in 10 mM Tris-HCl, pH 7.5. 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 7.5.

Calculate the amount of total RNA using the following formula:

Total RNA (µg) = OD260 × [40 µg/(1 OD260 × 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 the eluate was diluted to 500 µl in 10 mM Tris-HCl, pH 7.5. An OD_{260} of 0.188 was obtained. The amount of RNA in the sample is determined as shown below:

Total RNA (µg) =

$0.188 \times [40\ \mu g/(1\ OD_{260} \times 1\ ml)] \times 12.5 \times 0.15 = 14.1\ \mu g$

Continued on next page

RNA Quality

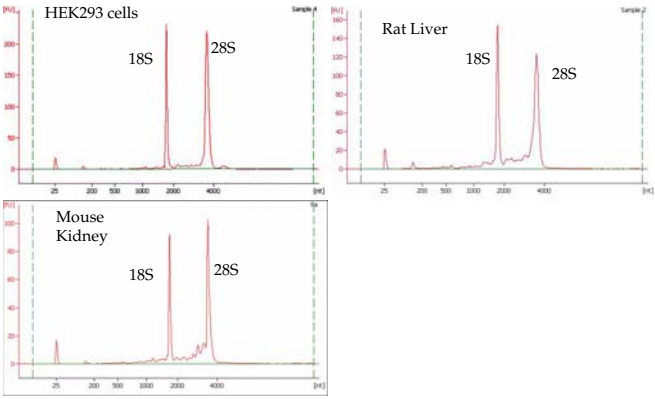
Typically, total RNA isolated using the PureLink™ RNA Micro Kit has an $OD_{260/280}$ of >1.8 when samples are diluted in Tris-HCl (pH 7.5). An $OD_{260/280}$ of >1.8 indicates that RNA is reasonably clean of proteins and other UV chromophores that could either interfere with downstream applications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

Agarose gel electrophoresis of RNA isolated using the PureLink™ RNA Micro 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.

Bioanalyzer Analysis of RNA Quality

The quality of purified total RNA can also be analyzed using a bioanalyzer such as the Agilent 2100 bioanalyzer with an RNA LabChip®. In the examples below, the bioanalyzer was used to show the presence of 18S and 28S rRNA, as well as small RNA species in total RNA purified using the PureLink™ RNA Micro Kit.

Total RNA was purified from HEK293 cells, Rat liver, and Mouse Kidney using the protocols described in this manual. Aliquots of 2% of the final elution volumes were subjected to bioanalysis using the Agilent 2100 bioanalyzer.



Expected Results

Expected Yields

The following table lists the average yields of total RNA obtained from various samples using the PureLink™ RNA Micro Kit. RNA quantitation was performed using UV absorbance at 260 nm.

Sample type	Sample	Amount	Average Yield
Animal Cells	HeLa	5×10^5	7.5 µg
	HEK293	5×10^5	10 µg
Animal Tissue	Rat liver	5 mg	17 µg
	Rat brain	5 mg	2 µg
	Rat spleen	5 mg	13.1 µg
	Rat heart	5 mg	1.8 µg
	Rat testes	5 mg	7.1 µg

Troubleshooting

Introduction

Refer to the table below to troubleshoot any problems you may encounter with the PureLink™ RNA Micro Kit.

Problem	Cause	Solution
Clogged Homogenizer	Highly viscous lysate (*e.g.*, calf thymus)	Homogenize sample with rotor–stator homogenizer.
Clogged RNA PureLink™ Micro Kit Column	Incomplete homogenization or dispersal of precipitate after ethanol addition	Follow protocol guidelines for each sample type and amount. Clear homogenate and remove any particulate or viscous material by centrifugation and use only the supernatant for subsequent loading on to the RNA PureLink™ Micro Kit Column. Completely disperse any precipitate that forms after adding ethanol to the homogenate.
Low RNA yield	Incomplete lysis and homogenization	Ensure that 10 µl of 2-mercaptoethanol was added per 1 ml of Lysis Buffer. Perform all steps at room temperature unless directed otherwise. Decrease the amount of starting material used, or increase volume of Lysis Buffer. Use the proper homogenization methods according to recommendations in the sample-specific protocols. Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in the Lysis Buffer to achieve optimal lysis.
	Poor quality of starting material	The yield and quality of RNA isolated depends on the type and age of the starting material. Be sure to use fresh sample and process immediately after collection or freeze the sample at –80ºC or in liquid nitrogen immediately after harvesting.

Continued on next page

Problem	Cause	Solution
Low RNA yield, continued	Ethanol not added to Wash Buffer II	Be sure that ethanol was added to Wash Buffer II as directed on page 9.
	Incorrect elution conditions	Add RNase-free water and perform incubation for 1 minute before centrifugation. Follow the recommendations under **Elution Parameters** (page 10). To recover more RNA, perform a second elution step.
RNA degraded	RNA contaminated with RNase	Use RNase-free pipet tips with aerosol barriers. Change gloves frequently. Swipe automatic pipets with RNase AWAY™ solution after washing the PureLink™ Micro Kit Column with Wash Buffer I.
	Improper handling of sample from harvest until lysis	If not processed immediately, quick-freeze tissue immediately after harvesting and store at –80ºC or in liquid nitrogen. Frozen samples must remain frozen until Lysis Buffer was added. Perform the lysis quickly after adding Lysis Buffer.
	Tissue very rich in RNases (*e.g.*, rat pancreas)	RNA isolated from tissue rich in RNases may require the addition of RNase inhibitors/inactivators to protect the RNA from degradation, or use a larger volume of Lysis Buffer. Elute samples in 100% formamide. If the RNA is used for mRNA isolation of Northern blots, elute in 0.1% SDS.

Continued on next page

Problem	Cause	Solution
DNA contamination	Incomplete homogenization or incomplete dispersal of precipitate after ethanol addition	Follow protocol guidelines for each sample type and amount.
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified RNA	Traces of ethanol from the Wash Buffer II can inhibit downstream enzymatic reactions. Discard Wash Buffer II flow through. Place the PureLink™ Micro Kit Column into the Wash Tube and centrifuge the PureLink™ Micro Kit Column at maximum speed for 2-3 minutes to completely dry the cartridge.
	Presence of salt in purified RNA	Use the correct order of Wash Buffers for washing. Always wash the cartridge with Wash Buffer I followed by washing with Wash Buffer II.
Low $A_{260}/_{280}$ ratio	Sample was diluted in water; non-buffered water has variable pH (Wilfinger *et al.*, 1997)	Use 10 mM Tris-HCl (pH 7.5) to dilute sample for OD measurements.

Appendix

Technical Support

Web Resources

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

Contact Us

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

Corporate Headquarters:	**Japanese Headquarters:**	**European Headquarters:**
Invitrogen Corporation 5791 Van Allen Way Carlsbad, CA 92008 USA	Invitrogen Japan LOOP-X Bldg. 6F 3-9-15, Kaigan Minato-ku, Tokyo 108-0022	Invitrogen Ltd Inchinnan Business Park 3 Fountain Drive Paisley PA4 9RF, UK
Tel: 1 760 603 7200	Tel: 81 3 5730 6509	Tel: +44 (0) 141 814 6100
Tel (Toll Free): 1 800 955 6288	Fax: 81 3 5730 6519	Tech Fax: +44 (0) 141 814 6117
Fax: 1 760 602 6500	E-mail: jpinfo@invitrogen.com	E-mail: eurotech@invitrogen.com
E-mail: tech_support@invitrogen.com		

MSDS

MSDSs (Material Safety Data Sheets) are available on our website at **www.invitrogen.com/msds.**

Certificate of Analysis

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

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Notes

Notes

invitrogen™

Corporate Headquarters
Invitrogen Corporation
5791 Van Allen Way
Carlsbad, CA 92008
T: 1 760 603 7200
F: 1 760 602 6500
E: tech_support@invitrogen.com

For country-specific contact information, visit our web site at **www.invitrogen.com**