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PureLink™ RNA Mini Kit

Catalog No. 12183020 **Quantity: 10 preps** **Store at room temperature**

Contents and Storage

The components of the PureLink™ RNA Mini Kit are shipped at room temperature. Upon receipt, store all components at room temperature. The components included with the kit are listed in the table below. Sufficient reagents are provided to perform 10 preparations.

Components	Quantity
Lysis Buffer	20 mL
Wash Buffer I	10 mL
Wash Buffer II	4 mL
RNase–Free Water	3 mL
Spin Cartridges (with collection tubes)	10 each
Collection Tubes	10 each
Recovery Tubes	10 each

Description

The PureLink™ RNA Mini Kit provides a simple, reliable, and rapid method for isolating high-quality total RNA from a wide variety of samples, including animal and plant cells and tissue, blood, bacteria, yeast, and liquid samples. The purified total RNA is suitable for use in a variety of downstream applications (see page 4).

Note: This manual provides protocols for purifying RNA from animal and plant cells only. The RNA Mini Kit allows you to purify RNA from a variety of samples and using alternate protocols. **If you wish to purify RNA from other samples or use these alternate protocols, refer to the main RNA Mini Kit manual**, available for downloading from the product page web site at www.invitrogen.com or by contacting Technical Support.

System Overview

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). After homogenization, ethanol is added to the sample. The sample is then processed through a Spin Cartridge 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).

Kit Specifications

Cartridge Binding Capacity:	~1 mg nucleic acid
Cartridge Reservoir Capacity:	700 µL
Wash Tube Capacity:	2.0 mL
Centrifuge Compatibility:	Capable of centrifuging >12,000 × *g*
Elution Volume:	30 µL–3 × 100 µL (3 sequential elutions with 100 µL each)
RNA Yield	Varies with sample type and quality

General Guidelines

- The maximum RNA binding capacity of the PureLink™ RNA Mini Kit Spin Cartridge is ~1 mg. If you are processing samples that contain more than 1 mg of total RNA, divide the sample into aliquots such that each aliquot contains <1 mg total RNA for each Spin Cartridge used.
- For an optional protocol with on-column DNAse treatment, refer to the full PureLink™ RNA Mini Kit manual.
- Use disposable, individually wrapped, sterile plastic ware and sterile, disposable RNase-free pipette tips and 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.
- Always use proper microbiological aseptic techniques when working with RNA.
- When purifying total RNA from fresh samples, keep fresh cell and tissue samples on ice immediately after harvesting and quickly proceed to **Lysis and Homogenization** (page 2).
- When purifying total RNA from frozen samples, freeze samples immediately after collection in liquid nitrogen or on dry ice. Keep samples frozen at –80°C or in liquid nitrogen until proceeding to **Lysis and Homogenization** (page 2).
- Use RNase *AWAY*® Reagent (cat. no. 10328-011) to remove RNase contamination from work surfaces and non-disposable items such as centrifuges and pipettes used during purification.

Part no. 100004470 MAN0000773 Rev. Date: 1 Jul 2010

Caution

- 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 isothiocyanate, as reactive compounds and toxic gases are formed.
- 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.

Purifying RNA from Animal and Plant Cells

Materials Needed

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

- 2–mercaptoethanol
- 70% ethanol (in RNase-Free Water)
- 1.5 mL RNase-free microcentrifuge tubes
- PBS (>10^7 cells per sample)
- 96–100% ethanol
- Microcentrifuge capable of centrifuging 12,000 × g
- 15 mL RNase-free tubes (>10^7 cells per sample)
- RNase-free pipet tips
- Homogenizer, or RNase-free syringe (1 mL) with 18–21-gauge needle or, Rotor-stator homogenizer

Buffer Preparation and Parameters

Preparing Wash Buffer II with Ethanol

Before beginning lysis and homogenization, add 16 mL 96–100% ethanol to Wash Buffer II. Check the box on the Wash Buffer II label to indicate that ethanol was added. Store Wash Buffer II with ethanol at room temperature.

Preparing Lysis Buffer with 2-Mercaptoethanol

Before beginning lysis and homogenization, 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 used.*

Amount of Lysis Buffer Needed

Number of cells in your sample	Amount of Lysis Buffer Needed (prepared with 2-mercaptoethanol)
≤1×10^6	0.3 mL* (*Use 0.6 mL if using rotor-stator for lysis or homogenization)
1×10^6–5×10^6	0.6 mL
5×10^6–5×10^7	0.6 mL per 5×10^6 cells (*e.g.*, use 1.2 mL for 1×10^7 cells and 6.0 mL for 5×10^7 cells)

Lysis and Homogenization

≤5×10^6 Suspension Cells

1. Transfer cells to an RNase-free tube and centrifuge at 2,000 × *g* for 5 minutes at 4°C to pellet. Discard growth medium.
2. Add 0.3 **or** 0.6 mL Lysis Buffer with 2-mercaptoethanol to your sample (see table above for correct amounts).
3. Vortex at high speed until the cell pellet is completely dispersed and the cells appear lysed.
4. Proceed with **one** of the following homogenization options at room temperature:
   a. Transfer the lysate to a Homogenizer inserted in a Collection Tube and centrifuge at 12,000 × *g* for 2 minutes, **or**
   b. Pass the lysate 5–10 times through an 18- to 21-gauge needle attached to an RNase-free syringe, **or**
   c. Transfer the lysate to an RNase-free tube and homogenize using a rotor-stator homogenizer at maximum speed for at least 45 seconds. Centrifuge the homogenate at ~2,600 × *g* for 5 minutes, then transfer the supernatant to a clean RNase–free tube.
5. Proceed to **Binding, Washing, and Elution**, next page.

≤5×10^6 Monolayer Cells

1. Remove the growth medium from the cells, then add 0.3 or 0.6 mL Lysis Buffer with 2-mercaptoethanol to your sample (see table above for correct amounts).
2. Proceed with **one** of the following homogenization options at room temperature:
   a. Transfer the lysate to a Homogenizer inserted in a Collection Tube and centrifuge at 12,000 × g for 2 minutes, **or**
   b. Transfer the lysate to a 1.5 mL tube and pass 5–10 times through an 18- to 21-gauge needle attached to a syringe, **or**
   c. Transfer the lysate to an appropriately RNase-free tube and homogenize using a rotor-stator homogenizer at maximum speed for at least 45 seconds. Centrifuge the homogenate at ~2,600 × g for 5 minutes, then transfer the supernatant to a clean RNase-free tube.
3. Proceed to **Binding, Washing, and Elution**, next page.

Lysis and Homogenization, Continued

5×10^6–5×10^7 Suspension Cells

Note: This protocol uses a rotor-stator homogenizer. Use 15-mL RNase-free tubes to compensate for volume expansion.

1. Transfer cells to a 15-mL tube and centrifuge at 2,000 × *g* for 5 minutes at 4°C to pellet. Remove and discard the supernatant.
2. Add 0.6 mL Lysis Buffer with 2-mercaptoethanol per 5×10^6 cells to your sample (refer to previous page for amounts).
3. Vortex at high speed until the cell pellet is completely dispersed and the cells appear lysed.
4. Homogenize cells using a rotor-stator homogenizer at maximum speed for at least 45 seconds, then centrifuge the homogenate at ~2,600 × *g* for 5 minutes at room temperature then transfer the supernatant to a clean 15-mL RNase–free tube.
5. Proceed to **Binding, Washing, and Elution**, below

Frozen Cell Pellets

1. Transfer the frozen cell pellet to an RNase-free tube then **add** the appropriate volume of Lysis Buffer prepared with 2-mercaptoethanol to your sample (refer to the previous page for correct amounts).
2. Vortex at high speed until the cell pellet is completely dispersed and the cells appear lysed.
3. Proceed with **one** of the following homogenization options at room temperature:
   a. Transfer the lysate to a Homogenizer inserted in a Collection Tube and centrifuge at 12,000 × *g* for 2 minutes, **or**
   b. Pass the lysate 5–10 times through an 18- to 21-gauge needle attached to an RNase-free syringe, **or**
   c. Transfer the lysate to an RNase-free tube and homogenize using a rotor-stator homogenizer at maximum speed for at least 45 seconds. Centrifuge the homogenate at ~2,600 × *g* for 5 minutes, then transfer the supernatant to a clean RNase-free tube.
4. Proceed to **Binding, Washing, and Elution**, below.

Binding, Washing and Elution

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

1. Add one volume 70% ethanol to each volume of cell homogenate (prepared as described above).

   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 the Spin Cartridge (with the Collection Tube).
4. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through, and reinsert the Spin Cartridge into the same Collection Tube.

   Note: When extracting RNA from larger quantities of starting material the homogenate may become viscous, requiring additional centrifugation (up to 15 minutes) for the sample to pass completely through the column.
5. **Repeat** Steps 3–4 until the entire sample has been processed.
6. Add 700 µL Wash Buffer I to the Spin Cartridge.
7. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through **and** the Collection Tube. Place the Spin Cartridge into a **new** Collection Tube.
8. Add 500 µL Wash Buffer II with ethanol (previous page) to the Spin Cartridge.
9. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through.
10. Repeat Steps 8–9 **once.**
11. Centrifuge the Spin Cartridge at 12,000 × *g* for 1–2 minutes to dry the membrane with attached the RNA. **Discard** the Collection Tube and insert the Spin Cartridge into a Recovery Tube.
12. Add 30 µL–100 µL RNase–Free Water to the center of the Spin Cartridge.
13. Incubate at room temperature for 1 minute.
14. Centrifuge the Spin Cartridge for 2 minutes at ≥12,000 × *g* at room temperature to elute the RNA from the membrane into the Recovery tube.

    Note: If the expected RNA yield is >100 µg, perform 3 sequential elutions of 100 µL each. Collect the eluates in a single tube.
15. Store your purified RNA or proceed to downstream application of choice.

Storage and Downstream Applications 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.

You may use the purified total RNA for qRT-PCR, northern blotting, nuclease protection assays, RNA amplification for microarray analysis, cDNA library preparation after poly(A)+ selection, or any desired downstream application.

If highly pure RNA without genomic DNA contamination is required, perform DNase I treatment after purification (refer to the PureLink™ RNA Mini Kit manual available from our web site at www.invitrogen.com for details).You can determine the quality and quantity of the purified RNA using UV absorbance at 260 nm or with the Quant-iT™ RNA Assay Kit (Cat. no. Q33140).

Accessory Products

A large selection of products for RT-PCR, qRT-PCR, microarray analysis, and reverse transcription is available separately from Invitrogen. For more information, visit our web site at www.invitrogen.com or contact Technical Support.

Troubleshooting

Observation	Cause	Solution
Low RNA yield	Incomplete lysis and homogenization	Use the appropriate method for lysate preparation based on your starting material as described on page 2. Decrease the amount of starting material used. 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. Use fresh sample and process immediately after collection or freeze the sample at –80°C or in liquid nitrogen immediately after harvesting.
	Clogged RNA Spin Cartridge	Clear the homogenate and remove particulate or viscous material by centrifugation. Use only the supernatant for subsequent loading onto the Spin Cartridge.
	Ethanol not added to Wash Buffer II	Add ethanol to Wash Buffer II before use (page 2).
	Incorrect elution conditions	Add RNase–Free Water (30–100 µL) and perform incubation for 1 minute before centrifugation. To recover more RNA, be sure to use up to 3 sequential elutions of 100 µL each (3 × 100 µL) Elution Buffer (refer to protocol on page 3).
RNA degraded	RNA contaminated with RNase	Follow the guidelines on page 2 to prevent RNase contamination.
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 Spin Cartridge into the Recovery Tube and centrifuge at 12,000 × g for 1–2 minutes to completely dry the cartridge.
	Presence of salt in purified RNA	Use the correct order of Wash Buffers for washing. Always wash with Wash Buffer I followed by washing with Wash Buffer II.
Low A_{260}/A_{280} ratio	Sample was diluted in water	Use 10 mM Tris–HCl (pH 7.5) to dilute sample for OD measurements.

References

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