PureLink™ RNA Mini Kit (Protocol for RNA clean-up and purification from liquid samples)

invitrogen

QUICK REFERENCE **Pub. No.** MAN0019349 **Rev.** A.0

Contents

Catalog Number 12183020

Components	Amount	Storage
Lysis Buffer	20 mL	Room temperature
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 Buffer	10 each	

Product 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.
- Isolate up to 1 mg of nucleic acid.

Required materials

- 2–mercaptoethanol
- 100% ethanol
- (*Optional*) PureLink™ DNase Set (Cat. No. 12185010)
- Microcentrifuge capable of 12,000 × *g*
- 1.5 mL RNase-free microcentrifuge tubes
- RNase-free pipet tips

Online resources

- Visit our product pages for protocols, safety, and additional product information.
- Go online to view related PureLink™ products.
- For support, visit thermofisher.com/support.

Before first use of the kit

Prepare Wash Buffer II

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.

(*Optional*) Prepare PureLink™ DNase Mixture

Add the following components to a clean, RNase-free microcentrifuge tube. Prepare 80 µL for each sample to be processed. Store PureLink™ DNase Mixture at –20°C.

Component	Volume
10X DNase I Reaction Buffer	8 µL
Resuspended DNase (~3U/µL)	10 µL
RNase free water	62 µL

Before each use of the kit

Prepare fresh Lysis Buffer

Add 10 µL of 2–mercaptoethanol for each 1 mL of Lysis Buffer needed for the purification procedure in a RNase-free tube.

Use 1 volume of Lysis Buffer for every 0.2 mL of whole blood (or fraction thereof).

Troubleshooting

For detailed troubleshooting instructions see the PureLink™ RNA Mini Kit User Guide at thermofisher.com or contact Technical Support.

Limited product warranty and licensing information

Manufacturer: Life Technologies Corporation | 2130 Woodward Street | Austin, TX 78744

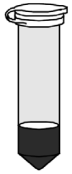
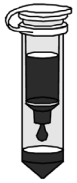
ThermoFisher SCIENTIFIC

Protocol for purification of RNA from liquid samples

This protocol describes how to purify or desalt one sample of total RNA from ≤1.2 mL of liquid sample (e.g., enzymatic reaction, upper phase from TRIzol™ reagent protocol in ethanol) on one column using the PureLink™ RNA Mini Kit. For detailed instructions see the PureLink™ RNA Mini Kit User Guide at thermofisher.com or contact Technical Support.

Important guidelines

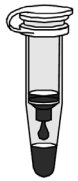
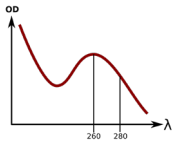
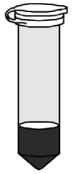
- Use proper RNA handling techniques when working with RNA.

Step		Action	
1		**Add Lysis Buffer**	a. Add one volume of liquid sample (≤1.2 mL) to a 1.5 mL RNase–free microcentrifuge tube. b. Add one volume of Lysis Buffer with 2-mercaptoethanol to the sample. c. Add one volume of 100% ethanol to the sample. d. Mix by vortexing or pipetting up and down 5 times.
2		**Bind RNA**	a. Transfer the sample (≤700 µL) to a Spin Cartridge (with Collection Tube). b. Centrifuge at 12,000 × *g* for 15 sec at room temperature. Discard the flow-through, and reinsert the Spin Cartridge into the same Collection Tube. c. Repeat steps a–b until the entire sample is processed.

Protocol for purification of RNA from liquid samples

Step		Action	
		No DNase treatment	On-column DNase treatment
3 	**Wash RNA**	a. (*for cytoplasmic RNA extracts*) Add 700 µL Wash Buffer I to the Spin Cartridge. Centrifuge at 12,000 × g for 15 sec at room temperature. Discard the flow-through and the Collection Tube. Place the Spin Cartridge into a new Collection Tube. b. Add 500 µL Wash Buffer II with ethanol to the Spin Cartridge. c. Centrifuge at 12,000 × g for 15 sec at room temperature. d. Discard the flow-through and reinsert the Spin Cartridge in the same Collection Tube. e. Repeat steps b–d one more time.	a. Add 350 µL Wash Buffer I to the Spin Cartridge. b. Centrifuge at 12,000 × g for 15 sec at room temperature. c. Discard the flow-through and the Collection Tube. Place the Spin Cartridge into a new Collection Tube. d. Add 80 µL PureLink™ DNase Mixture onto the surface of the Spin Cartridge membrane. e. Incubate at room temperature for 15 min. f. Add 350 µL Wash Buffer I to the Spin Cartridge. g. Centrifuge at ~2,600 × *g* for 5 min at room temperature. h. Discard the flow-through and the Collection Tube. Place the Spin Cartridge into a new Collection Tube. i. Add 500 µL Wash Buffer II with ethanol to the Spin Cartridge. j. Centrifuge at 12,000 × g for 15 sec at room temperature. k. Discard the flow-through and reinsert the Spin Cartridge in the same Collection Tube. l. Repeat steps i–k one more time.

Protocol for purification of RNA from liquid samples

Step		Action
4	 **Elute RNA**	a. Centrifuge the Spin Cartridge with Collection Tube at 12,000 × *g* for 1 min at room temperature. b. Discard the Collection Tube and insert the Spin Cartridge into a Recovery Tube. c. Add 30 µL to 3 × 100 µL RNase-Free Water to the center of the Spin Cartridge. d. Incubate at room temperature for 1 min. e. Centrifuge at 12,000 × g for 2 min at room temperature. Note: Collect all eluates into the same tube when performing serial elution.
5	 **Analyze RNA yield and quality**	Determine the quantity and quality of the purified total RNA using any of the following techniques (See the PureLink™ RNA Mini Kit User Guide for details). ▪ UV absorbance at 260 nm ▪ Fluorescence microplate reader with Quant-iT™ RiboGreen™ RNA Assay Kit
6	 **Store RNA**	▪ Keep purified RNA on ice if using the RNA within a few hours of isolation. ▪ Store purified RNA at –80°C or long-term storage.