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*
- 1.5–15 mL round-bottomed RNase-free 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 0.2 mL 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

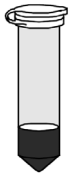
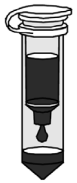
Manufacturer: Life Technologies Corporation | 2130 Woodward Street | Austin, TX 78744

Protocol for purification of RNA from whole blood

This protocol describes how to purify one sample of total RNA from ≤ 0.2 mL of fresh or frozen whole blood 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

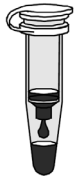
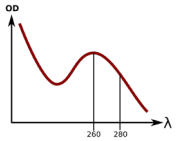
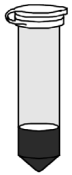
- Use proper RNA handling techniques when working with RNA.
- Collect whole blood in the presence of anticoagulants such as EDTA or sodium citrate and store at 4ºC until ready for use.
- Freshly drawn blood can be used without anticoagulants.
- Processing frozen blood may result in lower yield and quality of RNA.

Step		Action
1 	**Lyse cells**	a. Add ≤ 0.2 mL of whole blood sample to a 1.5 mL RNase–free microcentrifuge tube. b. Add 0.2 mL of Lysis Buffer with 2-mercaptoethanol to the sample. c. Vortex the lysate to completely disrupt and lyse the blood cells. d. Centrifuge at 12,000 × *g* for 2 min. e. Transfer the supernatant to a clean 1.5 mL RNase–free microcentrifuge tube.
2 	**Bind RNA**	a. Add 1.5 volumes of 100% ethanol to the sample. b. Disperse any precipitate by vortexing or pipetting up and down several times. c. Transfer the sample (including any remaining precipitate) to a Spin Cartridge (with Collection Tube). d. 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.

Protocol for purification of RNA from whole blood

Step		Action	
		No DNase treatment	On-column DNase treatment
3 	**Wash RNA**	a. Add 700 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 500 µL Wash Buffer II with ethanol to the Spin Cartridge. e. Centrifuge at 12,000 × g for 15 sec at room temperature. f. Discard the flow-through and reinsert the Spin Cartridge in the same Collection Tube. g. Repeat steps d–f 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 whole blood

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.
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.