PureLink™ RNA Mini Kit (Protocol for purification from animal and plant cells)

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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
- PBS
- (*Optional*) PureLink™ DNase Set (Cat. No. 12185010)
- 1.5 mL RNase-free microcentrifuge tubes
- Microcentrifuge capable of 12,000 × g
- 15 mL RNase-free tubes
- RNase-free pipet tips
- Homogenizer, or 1 mL RNase-free syringe with 18–21-gauge needle, or rotor-stator homogenizer

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.

For Research Use Only. Not for use in diagnostic procedures.

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

Cells/sample	Required Lysis Buffer volume/sample
≤1 × 10^6	0.3 mL (0.6 mL if using a rotor-stator for lysis or homogenization)
1 × 10^6– 5 × 10^6	0.6 mL
5 × 10^6– 5 × 10^7	0.6 mL for every 5 × 10^6 cells

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

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ThermoFisher SCIENTIFIC

Protocol for purification of RNA from animal and plant cells

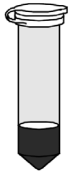
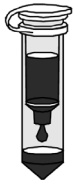
This protocol describes how to purify one sample of total RNA on one column using the PureLink™ RNA Mini Kit. For detailed instructions see the PureLink™ RNA Mini Kit User Guide at thermofisher.com or contact Technical Support.

Important guidelines

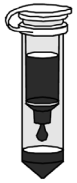
- For samples with more than >1 mg of total RNA, divide the sample into aliquots containing <1 mg of total RNA for each Spin Cartridge used.
- Use proper RNA handling techniques when working with RNA.
- When purifying total RNA from fresh samples, keep fresh cell and tissue samples on ice immediately after harvesting and quickly proceed to homogenization step.

Recommended homogenization method

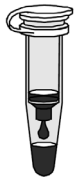
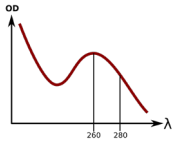
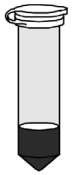
Cells/sample	Homogenizer type
≤5 × 10^6	Homogenizer, or syringe and needle, or rotor-stator homogenizer
5 × 10^6– 5 × 10^7	Rotor-stator homogenizer

Step		Action		
		Suspension cells	Monolayer cells	Frozen cell pellet
1 	**Harvest cells**	a. Transfer cells to an RNase-free tube and centrifuge at 2,000 × g for 5 min at 4°C. Discard growth medium. b. Add the appropriate volume of Lysis Buffer with 2-mercaptoethanol to your sample (see **Prepare fresh Lysis Buffer**). c. Vortex at high speed until the cell pellet is completely dispersed and the cells appear lysed.	a. Remove the growth medium from the cells. b. Add the appropriate volume of Lysis Buffer with 2-mercaptoethanol to your sample (see **Prepare fresh Lysis Buffer**).	a. Place the frozen cell pellet into an appropriately sized RNase-free tube. b. Add the appropriate volume of Lysis Buffer with 2-mercaptoethanol to your sample (see **Prepare fresh Lysis Buffer**). c. Vortex at high speed until the cell pellet is completely dispersed and the cells appear lysed.
2 	**Homogenize cells**	a. Perform one of the following homogenization options at room temperature (see **Recommended homogenization method**): ▪ Transfer the lysate to a Homogenizer inserted in a Collection Tube and centrifuge at 12,000 × *g* for 2 min. ▪ 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. ▪ Transfer the lysate to an appropriately RNase-free tube and homogenize using a rotor-stator homogenizer at maximum speed for >45 sec. Centrifuge the homogenate at ~2,600 × *g* for 5 min, then transfer the supernatant to a clean RNase-free tube.		

Protocol for purification of RNA from animal and plant cells

<table>
<tr><th colspan="2">Step</th><th colspan="2">Action</th></tr>
<tr><td>3</td><td>  </td><td>Bind RNA</td><td colspan="2">a. Add 1.5 volumes of 100% ethanol and cell lysate to an appropriately sized RNase-free tube.
b. Vortex to mix thoroughly and to disperse any visible precipitate that may form after adding ethanol.
c. Transfer up to 700 µL of the sample (including any remaining precipitate) to the Spin Cartridge (with the Collection Tube).
d. 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.
e. Repeat Steps c–d until the entire sample has been processed.</td></tr>
<tr><td rowspan="2">4</td><td rowspan="2">  </td><td rowspan="2">Wash RNA</td><td>No DNase treatment</td><td>On-column DNase treatment</td></tr>
<tr><td>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.</td><td>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.</td></tr>
</table>

Protocol for purification of RNA from animal and plant cells

Step		Action
5	 **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.
6	 **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
7	 **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.