

**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
- 10% SDS (in RNase-free water)
- Lysozyme solution
- (Optional) PureLink™ DNase Set (Cat. No. 12185010)
- Microcentrifuge capable of 12,000 × g
- 1.5 mL RNase-free microcentrifuge tubes
- RNase-free pipet tips
- Homogenizer (Cat. No. 12183026), or 1 mL RNase-free syringe with 18-21 gauge needles, 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](http://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 Lysozyme Solution**

Lysozyme Solution consists of 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, and 10 mg/mL lysozyme in RNase-free water.

Use 100 µL of fresh Lysozyme Solution for every 1 × 10<sup>9</sup> bacterial cells

**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 350 µL of fresh Lysis Buffer for every 1 × 10<sup>9</sup> bacterial cells

**Troubleshooting**

For detailed troubleshooting instructions see the [PureLink™ RNA Mini Kit User Guide](#) at [thermofisher.com](http://thermofisher.com) or contact Technical Support.

 **Limited product warranty and licensing information**

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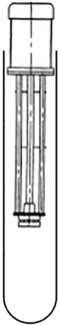
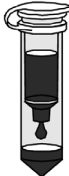
## Protocol for purification of RNA from bacterial cells

This protocol describes how to purify one sample of total RNA from  $1 \times 10^9$  bacterial cells on one column using the PureLink™ RNA Mini Kit. For detailed instructions see the PureLink™ RNA Mini Kit User Guide at [thermofisher.com](http://thermofisher.com) or contact Technical Support.


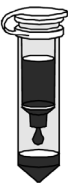


### Important guidelines

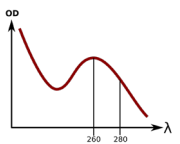
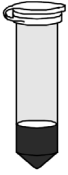
- Use proper RNA handling techniques when working with RNA.
- Fast and complete disruption of cells during the homogenization step is important to prevent RNA degradation.
- For best results, use cells in log phase growth to perform the purification procedure.
- Ensure the pH of the lysozyme buffer is correct. Lower pH may result in reduced RNA yield.

Step		Action
<p data-bbox="76 831 98 855"><b>1</b></p> 	<p data-bbox="454 831 689 855"><b>Homogenize cells</b></p>	<ol style="list-style-type: none"> <li>Centrifuge <math>\leq 1 \times 10^9</math> bacterial cells at <math>500 \times g</math> for 5 min at <math>4^\circ\text{C}</math>. Discard the supernatant.</li> <li>Add 100 <math>\mu\text{L}</math> of Lysozyme Solution to the cell pellet and resuspend by vortexing.</li> <li>Add 500 <math>\mu\text{L}</math> of 10% SDS solution and resuspend by vortexing.</li> <li>Incubate for 5 min at room temperature.</li> <li>Add 350 <math>\mu\text{L}</math> of Lysis Buffer with 2-mercaptoethanol to the sample and resuspend by vortexing.</li> <li>Add the sample drop-wise onto the dry ice in the mortar. Grind the mixture with the pestle until the dry ice evaporates and the lysate starts to melt.</li> <li>Homogenize the lysate using one of the following methods. <ul style="list-style-type: none"> <li>▪ Transfer the lysate to a Homogenizer inserted in an RNase-free tube. Centrifuge at <math>12,000 \times g</math> for 2 min at room temperature. Remove the Homogenizer when done.</li> <li>▪ Transfer the lysate to a 1.5 mL RNase-free tube and pass the lysate through an 18–21-gauge needle 5 times. Centrifuge at <math>12,000 \times g</math> for 2 min at room temperature, then transfer the supernatant to a clean RNase-free tube.</li> <li>▪ Transfer the lysate to a 4–15 mL round bottomed microcentrifuge tube. Homogenize using a rotor-stator homogenizer at maximum speed for <math>\geq 45</math> sec. Centrifuge the homogenate at <math>\sim 2,600 \times g</math> for 5 min at room temperature, then transfer supernatant to clean RNase-free tube.</li> </ul> </li> </ol>
<p data-bbox="76 1294 98 1318"><b>2</b></p> 	<p data-bbox="510 1294 633 1318"><b>Bind RNA</b></p>	<ol style="list-style-type: none"> <li>Add 1.5 volumes of 100% ethanol and bacterial lysate to an appropriately sized RNase-free tube.</li> <li>Mix thoroughly by vortexing to disperse any visible precipitate.</li> <li>Transfer the sample (including any remaining precipitate) to a Spin Cartridge (with Collection Tube).</li> <li>Centrifuge at <math>12,000 \times g</math> for 15 sec at room temperature. Discard the flow-through, and reinsert the Spin Cartridge into the same Collection Tube.</li> <li>Repeat steps c–d until the entire sample is processed.</li> </ol>

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Step		Action	
		No DNase treatment	On-column DNase treatment
3	 <p><b>Wash RNA</b></p>	<ol style="list-style-type: none"> <li>Add 700 <math>\mu\text{L}</math> Wash Buffer I to the Spin Cartridge.</li> <li>Centrifuge at <math>12,000 \times g</math> for 15 sec at room temperature.</li> <li>Discard the flow-through and the Collection Tube. Place the Spin Cartridge into a new Collection Tube.</li> <li>Add 500 <math>\mu\text{L}</math> Wash Buffer II with ethanol to the Spin Cartridge.</li> <li>Centrifuge at <math>12,000 \times g</math> for 15 sec at room temperature.</li> <li>Discard the flow-through and reinsert the Spin Cartridge in the same Collection Tube.</li> <li>Repeat steps d–f one more time.</li> </ol>	<ol style="list-style-type: none"> <li>Add 350 <math>\mu\text{L}</math> Wash Buffer I to the Spin Cartridge.</li> <li>Centrifuge at <math>12,000 \times g</math> for 15 sec at room temperature.</li> <li>Discard the flow-through and the Collection Tube. Place the Spin Cartridge into a new Collection Tube.</li> <li>Add 80 <math>\mu\text{L}</math> PureLink™ DNase Mixture onto the surface of the Spin Cartridge membrane.</li> <li>Incubate at room temperature for 15 min.</li> <li>Add 350 <math>\mu\text{L}</math> Wash Buffer I to the Spin Cartridge.</li> <li>Centrifuge at <math>\sim 2,600 \times g</math> for 5 min at room temperature.</li> <li>Discard the flow-through and the Collection Tube. Place the Spin Cartridge into a new Collection Tube.</li> <li>Add 500 <math>\mu\text{L}</math> Wash Buffer II with ethanol to the Spin Cartridge.</li> <li>Centrifuge at <math>12,000 \times g</math> for 15 sec at room temperature.</li> <li>Discard the flow-through and reinsert the Spin Cartridge in the same Collection Tube.</li> <li>Repeat steps i–k one more time.</li> </ol>
4	 <p><b>Elute RNA</b></p>	<ol style="list-style-type: none"> <li>Centrifuge the Spin Cartridge with Collection Tube at <math>12,000 \times g</math> for 1 min at room temperature.</li> <li>Discard the Collection Tube and insert the Spin Cartridge into a Recovery Tube.</li> <li>Add 30 <math>\mu\text{L}</math> to 3 <math>\times</math> 100 <math>\mu\text{L}</math> RNase-Free Water to the center of the Spin Cartridge.</li> <li>Incubate at room temperature for 1 min.</li> <li>Centrifuge at <math>12,000 \times g</math> for 2 min at room temperature. Note: Collect all eluates into the same tube when performing serial elution.</li> </ol>	

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5	 <p><b>Analyze RNA yield and quality</b></p>	<p>Determine the quantity and quality of the purified total RNA using any of the following techniques (See the <a href="#">PureLink™ RNA Mini Kit User Guide</a> for details).</p> <ul style="list-style-type: none"><li>▪ UV absorbance at 260 nm</li><li>▪ Fluorescence microplate reader with Quant-iT™ RiboGreen™ RNA Assay Kit</li></ul>
6	 <p><b>Store RNA</b></p>	<ul style="list-style-type: none"><li>▪ Keep purified RNA on ice if using the RNA within a few hours of isolation.</li><li>▪ Store purified RNA at -80°C or long-term storage.</li></ul>