PureLink™ RNA Mini Kit (Protocol for purification of plant tissues)

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QUICK REFERENCE Pub. No. MAN0019350 Rev. B

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)
- 1.5–15 mL round-bottomed RNase-free tubes
- RNase-free pipet tips
- Microcentrifuge capable of 12,000 × *g*
- RNase-free scalpels and tweezers, petri dish (for fresh plant tissues)
- Pestle/mortar and liquid nitrogen (for frozen tissues or fresh fibrous tissues)
- Homogenizer (Cat. No. 12183026) 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.

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

Troubleshooting

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

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

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

ThermoFisher SCIENTIFIC

Protocol for purification of RNA from plant tissues

This protocol describes how to purify one sample of total RNA from 250 mg of fresh or frozen animal tissue 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.
- Fast and complete disruption of tissue during the homogenization step is important to prevent RNA degradation.

Guidelines for frozen tissue

- Keep frozen tissue at –80°C prior to lysis. Do not allow samples to thaw prior to lysis.
- Cool tubes for frozen samples on dry ice before placing frozen tissue in them.

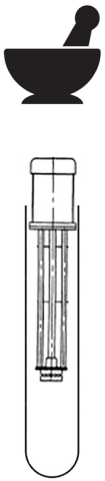
Before each use of the kit

Prepare fresh Lysis Buffer

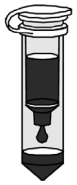
Add 10 µL of 2–mercaptoethanol for each 1 mL of Lysis Buffer needed for the purification procedure in a RNase-free tube.

Amount of tissue	Required Lysis Buffer volume
≤ 100 mg	0.5 mL [1]
100–200 mg	1.0 mL
200–250 mg	1.5 mL

[1] Prepare 0.6 mL if using rotor-stator for lysis or homogenization.

Step		Action	
		Frozen or fresh fibrous tissue	Soft tissue
1 	**Homogenize tissues**	a. (*for fresh fibrous tissue*) Cut tissue into small, ≤0.5 cm^2 pieces. b. Place samples in mortar, then add liquid nitrogen and grind tissue thoroughly using a pestle. c. Transfer tissue powder to an appropriately sized round-bottomed RNase-free microcentrifuge tube that has been cooled on liquid nitrogen. Allow liquid nitrogen to evaporate. d. Add the appropriate volume of Lysis Buffer with 2-mercaptoethanol to your sample. e. Vortex the lysate to completely disperse the sample. Incubate for 3 min at room temperature. f. Transfer ≤0.5 mL of lysate to a Homogenizer inserted in an RNase-free tube. Centrifuge at 12,000 × *g* for 2 min. Remove the Homogenizer when done. **Note**: Use additional homogenizers for each 0.5 mL of sample.	a. Cut tissue into small, ≤0.5 cm^2 pieces, then transfer tissue to an appropriately sized round-bottomed RNase-free microcentrifuge tube. b. Add the appropriate volume of Lysis Buffer with 2-mercaptoethanol to your sample. c. Homogenize the sample using a rotor-stator at maximum speed for at least 45 sec. d. Centrifuge at ~2,600 × *g* for 5 min at room temperature. e. Transfer the supernatant to a new RNase-free tube.

Protocol for purification of RNA from plant tissues

<table>
<tr><th colspan="2">Step</th><th colspan="2">Action</th></tr>
<tr><td>2</td><td>  </td><td>Bind RNA</td><td colspan="2">a. Add 1.5 volumes of 100% ethanol and tissue homogenate to an appropriately sized RNase-free tube.
b. Mix thoroughly by shaking or vortexing to disperse any visible precipitate.
c. Transfer up to 700 µL of 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.
e. Repeat steps c–d until the entire sample is processed.</td></tr>
<tr><td>3</td><td>  </td><td>Wash RNA</td><td>No DNase treatment
f. Add 700 µL Wash Buffer I to the Spin Cartridge.
g. Centrifuge at 12,000 × g for 15 sec 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 d–f one more time.</td><td>On-column DNase treatment
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>

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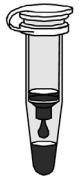
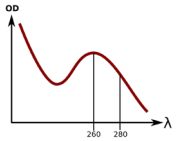
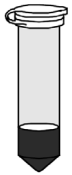
Step		Action
4	 **Elute RNA**	a. Centrifuge the Spin Cartridge with Collection Tube at 12,000 × *g* for 1 min at room temperature. b. Discard the Collection Tube and insert the Spin Cartridge into a Recovery Tube. c. Add 30 µL to 3 × 100 µL RNase-Free Water to the center of the Spin Cartridge. d. Incubate 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.