PureLink™ RNA Mini Kit (Protocol for purification from yeast cells)

invitrogen

QUICK REFERENCE Pub. No. MAN0019352 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)
- 1.5–15 mL round-bottomed RNase-free tubes
- RNase-free pipet tips
- Microcentrifuge capable of 12,000 × *g*
- Homogenizer (Cat. No. 12183026), or 1 mL RNase-free syringe with 18-21 gauge needles, or rotor-stator homogenizer
- Mortar (5 cm dia.), pestle, and dry ice (for mechanical lysis)
- Zymolyase (for enzymatic lysis)

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.

Lysis method	Required Lysis Buffer volume
Mechanical	0.5 mL
Enzymatic	0.2 mL

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

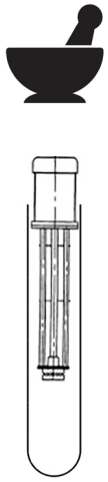
ThermoFisher SCIENTIFIC

Protocol for purification of RNA from yeast cells

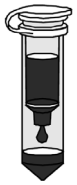
This protocol describes how to purify one sample of total RNA from 5 × 10^8 yeast cells on one column using the PureLink™ RNA Mini Kit. For detailed instructions see the PureLink™ RNA Mini Kit User Guide at thermofisher.com or contact Technical Support.

Important guidelines

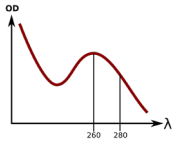
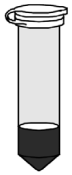
- Use proper RNA handling techniques when working with RNA.
- Fast and complete disruption of cells during the homogenization step is important to prevent RNA degradation.
- Grow yeast cells overnight so that they are at log phase prior to performing the purification procedure.
- Enzymatic lysis is a convenient procedure, but not suitable for kinetic experiments. Mechanical lysis is suitable for kinetic experiments but is a labor-intensive procedure.

Step		Action	
		Mechanical lysis	Enzymatic lysis
1 	**Homogenize cells**	a. Centrifuge ≤5 x 10^8 yeast cells at 500 × *g* for 5 min at 4°C. Discard the supernatant. b. Crush ~10 g of dry ice into powder using a RNase-free mortar and pestle. c. Add 500 µL of Lysis Buffer with 2-mercaptoethanol to the sample and resuspend. d. 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. e. Homogenize the lysate using one of the following methods. ▪ Transfer the lysate to a Homogenizer inserted in an RNase-free tube. Centrifuge at 12,000 × *g* for 2 min at room temperature. Remove the Homogenizer when done. ▪ 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 12,000 × *g* for 2 min at room temperature, then transfer the supernatant to a clean RNase-free tube. ▪ Transfer the lysate to a 4–15 mL round bottomed microcentrifuge tube. Homogenize using a rotor-stator homogenizer at maximum speed for ≥45 sec. Centrifuge the homogenate at ~2,600 × *g* for 5 min at room temperature, then transfer supernatant to clean RNase-free tube.	a. Prepare a digestion buffer with zymolyase according to manufacturer instructions, or use the digestion buffer provided by the supplier. **Note**: The amount of digestion buffer and zymolyase required depends on the type of yeast cells and the sample size. See the manufacturer instructions for guidance. b. Centrifuge ≤5 x 10^8 lyeast cells at 500 × *g* for 5 min at 4°C. Discard the supernatant. c. Add ≤100 µL of zymolyase digestion buffer to the yeast cell pellet. d. Incubate for 30–60 minutes at 30°C. The incubation time may vary depending on your yeast cell type and the enzyme used. e. Add 200 µL of Lysis Buffer with 2-mercaptoethanol to your sample. f. Vortex to mix thoroughly.

Protocol for purification of RNA from yeast cells

<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 yeast lysate to an appropriately sized RNase-free tube.
b. Mix thoroughly by vortexing to disperse any visible precipitate.
c. Transfer up to 500 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
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>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>

Protocol for purification of RNA from yeast cells

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.