PureLink™ RNA Mini Kit (Protocol for purification from animal tissues)

invitrogen

QUICK REFERENCE Pub. No. MAN0019346 Rev. B.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 Tubes	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
- 96–100% ethanol
- 70% ethanol
- (*Optional*) PureLink™ DNase Set (Cat. No. 12185010)
- RNase-free sample tubes (see page 2)
- Microcentrifuge capable of 12,000 × g
- RNase-free pipet tips
- RNase-free glass, Teflon, or plastic pestle
- Mortar and pestle with liquid nitrogen
- Homogenizer (Cat No. 12183026), 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 (~3 U/µL) [1]	10 µL
RNase free water	62 µL

[1] Resuspend the contents from a tube of PureLink™ DNase with 550 µL of RNase-free water.

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.

Tissue amount	Required Lysis Buffer volume/sample
≤10 mg	0.3 mL (0.6 mL if using a rotor-stator for lysis or homogenization)
10–30 mg	0.6 mL
30–200 mg	0.6 mL for every 30 mg of tissue

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

ThermoFisher SCIENTIFIC

Protocol for purification of RNA from animal tissues

This protocol describes how to purify one sample of total RNA from ≤ 200 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

- 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.

Troubleshooting

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

Recommended homogenization method

Choose a homogenization method appropriate for the sample type.

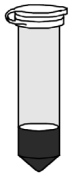
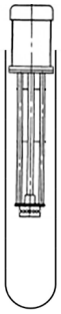
Tissue type	Sample size	Homogenizer type	Sample tube
Frozen or fresh fibrous	≤10 mg	Microcentrifuge pestle	1.5-mL microcentrifuge tube
		Rotor-stator homogenizer	4-mL round-bottom tube
	10–200 mg	Mortar and pestle	2-mL round-bottom tube
		Rotor-stator homogenizer	4-mL round-bottom tube
Fresh soft	≤100 mg	Microcentrifuge pestle	1.5-mL or2-mL round-bottom tube
		Rotor-stator homogenizer	4-mL round-bottom tube
	100–200 mg	Rotor-stator homogenizer	15-mL round-bottom tube
Stored in RNA*later* reagent	<100 mg	Rotor-stator homogenizer	15-mL round-bottom tube

Recommended elution volume

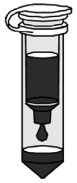
Choose an elution volume appropriate for the expected yield of RNA.

Elution volume	RNA yield quantity
30–100 µL	≤100 µg
2 × 100 µL	100–500 µL
3 × 100 µL	500–1,000 µL

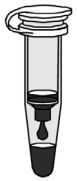
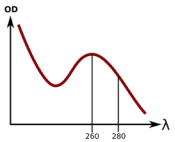
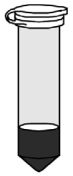
Protocol for purification of RNA from animal tissues

Step			Action
1		**Homogenize tissue (microfuge pestle)**	**If performing homogenization using microfuge pestle** a. Transfer the tissue into a pre-chilled 1.5-mL or 2-mL RNase-free round bottom-tube on ice (see **Recommended homogenization method**). b. Add the appropriate volume of Lysis Buffer with 2-mercaptoethanol to your sample (see **Prepare fresh Lysis Buffer**). c. Mince tissue with an RNase–free pestle using up/down and twisting movements in the tube until tissue is thoroughly disrupted and lysed. d. (*for ≤100 mg of fresh soft tissue*) Centrifuge the homogenate at ~1,200 × g for 2 min. e. (*for ≤100 mg of fresh soft tissue*) Transfer the supernatant to a clean RNase-free tube. f. Perform one of the following homogenization options at room temperature. ▪ Transfer the lysate to a Homogenizer (Cat No. 12183026) inserted in a RNase-free tube and centrifuge at 12,000 × *g* for 2 min. Remove the Homogenizer when done. ▪ Pass 5–10 times through an 18- to 21-gauge needle attached to a syringe. Centrifuge the homogenate at 12,000 × *g* for 2 min, then transfer the supernatant to a clean RNase-free tube.
		**Homogenize tissue (rotor-stator homogenizer)**	**If performing homogenization using rotor-stator homogenizer** a. Transfer the tissue into a pre-chilled 4-mL or 15-mL RNase-free tube on ice (see **Recommended homogenization method**). b. Add the appropriate volume of Lysis Buffer with 2-mercaptoethanol to your sample (see **Prepare fresh Lysis Buffer**). c. Homogenize the sample for ≥45 sec for ≥100 mg of tissue, or 30–40 sec for ≤100 mg of tissue. d. Centrifuge the homogenate at ~2,600 × *g* for 5 min. e. Transfer the supernatant to a clean RNase-free tube.
		**Homogenize tissue (mortar and pestle)**	**If performing homogenization using rotor-stator homogenizer** a. Place the tissue into a RNase-free mortar, then add liquid nitrogen and grind the tissue into powder. b. Transfer the powder into a 2-mL round-bottom RNase-free tube in liquid nitrogen, and allow the liquid nitrogen to evaporate. c. Add the appropriate volume of Lysis Buffer with 2-mercaptoethanol to your sample (see **Prepare fresh Lysis Buffer**). d. Perform one of the following homogenization options at room temperature. ▪ Transfer the lysate to a Homogenizer (Cat No. 12183026) inserted in a RNase-free tube and centrifuge at 12,000 × *g* for 2 min. Remove the Homogenizer when done. ▪ Pass 5–10 times through an 18- to 21-gauge needle attached to a syringe. Centrifuge the homogenate at 12,000 × *g* for 2 min, then transfer the supernatant to a clean RNase-free tube.

Protocol for purification of RNA from animal tissues

<table>
<tr><th colspan="3">Step</th><th colspan="2">Action</th></tr>
<tr><td>2</td><td>  </td><td>Bind RNA</td><td colspan="2">a. Add 1 volume of 70% ethanol to the tissue lysate in the 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>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 ~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 i–k one more time.</td></tr>
</table>

Protocol for purification of RNA from animal tissues

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 (See **Recommended elution volume**). 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 for long-term storage.