QUICK REFERENCE

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PureLink® RNA Mini Kit

Cat. nos. 12183018A, 12183025

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Directions for purifying RNA from animal and plant cells using the PureLink® RNA Mini Kit are described below.

For detailed instructions, and protocols for isolating RNA from tissue, blood, bacteria, yeast, liquid samples, and on-column DNAse treatment, download the manual from www.lifetechnologies.com or by contacting Technical Support.

General Guidelines

- Follow proper aseptic RNA handling techniques to prevent RNase contamination of reagents and RNA samples.
- Keep freshly harvested samples on ice and quickly proceed to Lysis and Homogenization, or freeze samples immediately after collection in liquid nitrogen or on dry ice and keep at –80°C for later use.
- Do not exceed the RNA binding capacity of the spin cartridge by adding samples containing more than 1 mg of total RNA.

- Both Lysis Buffer and Wash Buffer I contain guanidine isothiocyanate. Do not add bleach or acidic solutions directly to solutions or sample preparation waste containing guanidinium isothiocyanate, as reactive compounds and toxic gases are generated.
- Solutions containing ethanol are considered flammable. Use appropriate precautions when using this chemical.

Required Materials

- 96–100% ethanol
- 2–mercaptoethanol
- 70% ethanol (in RNase-Free Water)
- 1.5 mL RNase-free microcentrifuge tubes
- Homogenizer, RNase-free syringe (1 mL) with 18–21-gauge needle or, Rotor-stator homogenizer
- Microcentrifuge capable of centrifuging 12,000 × *g*
- PBS (for samples with >10^7 cells)
- 15 mL RNase-free tubes (for samples with >10^7 cells), RNase-free pipet tips

Buffer Preparation

- When using Wash Buffer II for the first time, add 60 mL 96–100% ethanol (Cat. no. 12183018A) or 300 mL 96–100% ethanol (Cat. no. 12183025). Mark the label to indicate that ethanol is already added.
- Prepare fresh Lysis Buffer containing 1% 2-mercaptoethanol. Add 10 µL 2-mercaptoethanol for every 1 mL Lysis Buffer (see page 2).

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Lysis and Homogenization

Required Volume of Lysis Buffer

Cell Number	Lysis Buffer Required for Each Sample
≤1 × 10^6	0.3 mL (0.6 mL if using a rotor-stator for lysis/homogenization)
1 × 10^6–5 × 10^6	0.6 mL
5 × 10^6–5 × 10^7	0.6 mL per 5 × 10^6 cells (e.g., use 1.2 mL for 1 × 10^7 cells)

≤5 × 10^6 Suspension Cells

1. Transfer the cells to an RNase-free tube and centrifuge at 2,000 × *g* for 5 min at 4°C to pellet. Discard the growth medium.
2. Add 0.3 or 0.6 mL Lysis Buffer with 2-mercaptoethanol to the sample (see table above for volume).
3. Vortex until the cell pellet is dispersed and the cells appear lysed.
4. Proceed to **Homogenization** below.

≤5 × 10^6 Monolayer Cells

1. Remove the growth medium from the cells, then add 0.3 or 0.6 mL Lysis Buffer with 2-mercaptoethanol (see table above for volume).
2. Vortex until the cell pellet is dispersed and the cells appear lysed.
3. Proceed to **Homogenization** below.

5 × 10^6– 5 × 10^7 Suspension Cells

1. Transfer cells to a 15-mL tube and centrifuge at 2,000 × *g* for 5 min at 4°C. Discard the supernatant.
2. Add 0.6 mL Lysis Buffer with 2-mercaptoethanol (see table above for volume).
3. Vortex until the cell pellet is dispersed and the cells appear lysed.
4. Homogenize at room temperature with a rotor-stator homogenizer (see **Homogenization** below).

Frozen Cell Pellets

1. Transfer cells to a 15-mL tube and add 0.6 mL Lysis Buffer with 2-mercaptoethanol (see table above for volume).
2. Vortex until the cell pellet is dispersed and the cells appear lysed.
3. Homogenize at room temperature with a rotor-stator homogenizer (see **Homogenization** below).

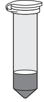
Homo- genization

1. Proceed with **one** of the following homogenization options at room temperature:
   - Transfer the lysate into a clean homogenization tube, and perform manual homogenization. Centrifuge the homogenate at 12,000 × *g* for 2 minutes.
   - Pass the lysate 5–10 times through an 18- to 21-gauge syringe needle.
   - Transfer the lysate into a clean tube, and homogenize using a rotor-stator homogenizer at maximum speed for ≥45 s. Centrifuge the homogenate at 26,000 × *g* for 5 minutes, then transfer the supernatant to a clean RNase-free tube.
2. Proceed to **RNA Purification**, next page.

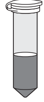
Binding, Washing, and Elution of RNA

1. Add one volume 70% ethanol to each volume of cell homogenate.
2. Vortex to mix thoroughly and to disperse any visible precipitate that may form after adding ethanol.
3. Transfer up to 700 µL of the sample (including any remaining precipitate) to the spin cartridge (with the collection tube).
4. 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.
5. Repeat Steps 3–4 until the entire sample has been processed.
6. Add 700 µL Wash Buffer I to the spin cartridge.
7. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through and the collection tube. Place the spin cartridge into a new collection tube.
8. Add 500 µL Wash Buffer II with ethanol to the spin cartridge.
9. Centrifuge at 12,000 × *g* for 15 seconds at room temperature. Discard the flow-through.
10. Repeat Steps 8–9 once.
11. Centrifuge the spin cartridge at 12,000 × *g* for 1–2 minutes to dry the membrane with bound RNA. Discard the collection tube and insert the spin cartridge into a recovery tube.
12. Add 30–100 µL RNase-free water to the center of the spin cartridge.
13. Incubate at room temperature for 1 minute.
14. Centrifuge the spin cartridge for 2 minutes at ≥12,000 × *g* at room temperature to elute the RNA from the membrane into the recovery tube. **Note:** If the expected RNA yield is >100 µg, perform 3 sequential elutions of 100 µL each. Collect the eluates in a single tube.
15. Store your purified RNA or proceed to downstream application.

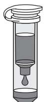
Lyse and homogenize sample in Lysis Buffer with 2-mercaptoethanol



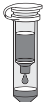
Add ethanol, mix thoroughly



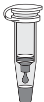
Add sample to Spin Cartridge to bind RNA to membrane



Wash sample 3X



Elute RNA into Recovery Tube



RNA Storage

Store the purified RNA on ice for immediate use. For long-term storage, keep the purified RNA at –80°C.

Perform DNase I treatment after purification (refer to the PureLink® RNA Mini Kit manual) to assure highly pure RNA without genomic DNA contamination.

Determine the quality and quantity of your RNA by UV absorbance at 260 nm.

Troubleshooting

Observation	Cause	Solution
Low RNA yield	Incomplete lysis and homogenization	Use the appropriate method for lysate preparation based on amount of starting material (see page 2). Decrease the amount of starting material used. Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in the Lysis Buffer to achieve optimal lysis.
	Poor quality of starting material	The yield and quality of RNA isolated depends on the type and age of the starting material. Use fresh sample and process immediately after collection or freeze the sample at –80°C or in liquid nitrogen immediately after harvesting.
	Clogged RNA Spin Cartridge	Clear the homogenate and remove particulate or viscous material by centrifugation. Use only the supernatant for subsequent loading onto the spin cartridge.
	Ethanol not added to Wash Buffer II	Add ethanol to Wash Buffer II before use (see page 1).
	Incorrect elution conditions	Add RNase-Free Water (30–100 µL) and incubate for 1 min before centrifugation. To recover more RNA, be sure to use up to 3 sequential elutions of 100 µL each (3 × 100 µL) Elution Buffer (refer to protocol on page 3).
RNA degraded	RNA contaminated with RNase	Use proper aseptic RNA handling techniques. Use RNase-free plasticware, and wear disposable gloves. Remove RNase contamination from work surfaces and non-disposable items with RNase AWAY® Reagent (Cat. no. 10328-011).
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified RNA	Traces of ethanol from Wash Buffer II can inhibit downstream enzymatic reactions. Discard Wash Buffer II flow through. Place the spin cartridge into the recovery tube and centrifuge at 12,000 × *g* for 1–2 min to completely dry the cartridge.
	Presence of salt in purified RNA	Use Wash Buffers in the correct order. Always wash with Wash Buffer I followed by Wash Buffer II.
Low A_{260}/A_{280} ratio	Sample was diluted in water	Use 10 mM Tris-HCl (pH 7.5) to dilute sample for OD measurements.

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