PureLink™ RNA Mini Kit

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 >107 cells)
- 15 mL RNase-free tubes (for samples with >107 cells), RNase-free pipet tips

Buffer Preparation

When using Wash Buffer II for the first time, add 60 mL 96–100% ethanol or 300 mL 96–100% ethanol. 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.

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 × 106 cells (e.g., use 1.2 mL for 1 × 107 cells)

Lysis and Homogenization

≤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).

Homogenization

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.

RNA Purification

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.

Storage

Store the purifi ed 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.

Source: ThermoFisher Scientific