TOOLSharp RNA Extractor

Cat. no. TTD-NRNA200
Storage: Room temperature for 1 year
Product Size: 200 mL

Introduction

The TOOLSharp RNA Extractor is the most efficient reagent for isolating total RNA from samples of human, animal, plant, bacterial, and viral origin. This unique reagent provides higher yield and quality of isolated RNA than the traditional reagents based on the single-step method. The TOOLSharp RNA Extractor isolates pure and undegraded RNA that is ready for use without DNase treatment. The TOOLSharp RNA Extractor separates RNA from other molecules in a single step based on the interaction of phenol and guanidine with cellular components. No chloroform-induced phase separation is necessary to obtain pure RNA. A biological sample is homogenized or lysed in the TOOLSharp RNA Extractor. DNA, proteins, polysaccharides, and other molecules precipitate out of the homogenate or lysate with the addition of water and its removal through centrifugation. The pure RNA is isolated from the resulting supernatant through alcohol precipitation, followed by elution.

Features

1. The isolation procedure can be completed in less than 1 h.
2. The isolated RNA is ready for use in RT-PCR, qRT-PCR, microarrays, polyA+ selection, northern blotting, RNase protection assay, and other molecular biology applications.
3. No chloroform-induced phase separation is necessary to obtain pure RNA.
4. The procedure, including centrifugation, is performed at room temperature.

Important Notes

1. This protocol yields all classes of RNA in one fraction, including large nuclear RNA, rRNA, mRNA, small RNA, and microRNA down to 10 bases.
2. The isolation is performed at room temperature and centrifuged at 4–28 °C.

Reagents Required by the User

1. Ethanol
2. Isopropanol
3. 4-bromoanisole (BAN, optional)
4. RNase-free water

Workflow

1. Homogenization: 1 mL TOOLSharp RNA Extractor per 100 mg tissue or 10^7 cells.
2. DNA or protein precipitation: homogenate + 0.4 mL water, wait 5–15 min, 12,000 × g 15 min.
3. BAN purification (optional): 1 mL supernatant + 5 µL 4-bromoanisole, wait 3–4 min, 12,000 ×g 10 min.
4. RNA precipitation: supernatant + 1 volume isopropanol, wait 15 min, 12,000 ×g 10 min.
5. RNA washes: 0.8 mL 75% ethanol, 4000 ×g 3 min; wash twice.
6. RNA solubilization: RNase-free water and stored at −70 °C.

An optional purification step using 4-bromoanisole (BAN) can be used to further eliminate DNA contamination.

Protocol

1. Homogenization
   A. Tissues

      Add 1 mL of TOOLSharp RNA Extractor per 50–100 mg of tissue to the sample and homogenize using a homogenizer (e.g., liquid nitrogen and a mortar are recommended).

   B. Cells

      Cells grown in monolayer should be lysed in a culture dish by adding the TOOLSharp RNA Extractor. Remove culture medium and add at least 1 mL of the reagent per 3.5 cm culture dish (10 cm^2). Pass the lysate through a pipette several times to ensure lysis. Cells grown in suspension should be sedimented first and then lysed by adding the TOOLSharp RNA Extractor. Add at least 1 mL of TOOLSharp RNA Extractor per 10^7 cells and lyse cells by repeated pipetting. Washing cells before the addition of the TOOLSharp RNA Extractor is not recommended, as it might cause RNA degradation. For cells grown in monolayer, use the amount of the TOOLSharp RNA Extractor based on the area of the culture dish and not the cell number. An insufficient amount of the TOOLSharp RNA Extractor will result in DNA contamination of the isolated RNA.

2. DNA, protein, and polysaccharide precipitation

Add 0.4 mL of ddH2O into the homogenate or lysate (with 1 mL of TOOLSharp RNA Extractor from step1). Vortex vigorously for 15 seconds and incubate for 5–15 min. Samples with 100 mg tissue/mL TOOLSharp RNA Extractor require “15-min incubation” at room temperature. Centrifuge whole lysate at 12,000 g for 15 min. After centrifugation, DNA, proteins, and most polysaccharides form a semisolid pellet at the bottom of the tube. The RNA remains soluble in the supernatant. Transfer 1 mL of the supernatant (75% of total supernatant volume, leave approximately 0.4 mL solution) to a new tube, leaving a layer of the supernatant above the DNA or protein pellet. Centrifugation at this and other steps of the protocol can be performed at 4–28 °C.

3. Phase separation (optional)

   A phase separation step can be incorporated into the basic protocol for total RNA isolation. This additional step is beneficial for samples with a high content of DNA or extracellular material. Add 5 µL (0.5% of the supernatant volume) of 4-bromoanisole to 1 mL of the transferred supernatant. Shake the resulting mixture for 15 seconds, store it for 3–5 min, and centrifuge it at 12,000 g for 10 min at 4–25 °C. After centrifugation, residual DNA, protein, and polysaccharide precipitate accumulates in the organic phase at the bottom of a tube, whereas the RNA remains soluble in the supernatant. The 4-bromoanisole cannot be substituted with bromochloropropane or chloroform.

4. Precipitation of total RNA

   Transfer the RNA-containing supernatant obtained in either Step 2 or 3 mentioned earlier to a new tube. Precipitate RNA by mixing 1 mL of the supernatant with 1 mL of isopropanol. Store samples for 10 min and centrifuge at 12,000 g for 10 min. In most cases, a white pellet of RNA precipitate forms at the bottom of the tube.

5. RNA elution

   Wash the RNA by mixing the pellet with 0.8 mL of 75% ethanol (v/v) and then centrifuge the tube twice at 4000 g for 3 min. Remove ethanol using a micropipette. Dissolve the RNA pellet, without drying, in RNase-free water and store at −70 °C. The isolated RNA has a 260/280 ratio of 1.7 to 2.1 and a 260/230 ratio of 1.6 to 2.3.