Azol® *NC* RNA Isolation Reagent

Cat. AzolNC.200
Qty. 200 ml

ARROWTEC

Content and Storage

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Description

Arrowtec Azol® NC RNA Isolation reagent is the most effective reagent for isolation of total RNA from samples of human, animal, plant, bacterial and viral origin. This unique reagent provides higher yield and quality of isolated RNA than traditional reagents based on the single-step method. Azol® NC RNA Isolation reagent isolates pure and undegraded RNA that is ready for the following application without DNase treatment. Azol® NC RNA Isolation reagent 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 Azol® NC RNA Isolation reagent. DNA, proteins, polysaccharides and other molecules precipitate out of the homogenate/ lysate by addition of water and removing it by centrifugation. The pure RNA is isolated from the resulting supernatant by alcohol precipitation, followed by washing and solubilization.

Key Features

- The isolation procedure can be completed in less than one hour. The isolated RNA is ready for use in RT-PCR, qRT-PCR, microarrays, poly A+ selection, northern blotting, RNase protection assay and other molecular biology applications.
- No chloroform-induced phase separation is necessary to obtain pure RNA.
- The Azol® NC RNA Isolation reagent procedure is performed at room temperature, including centrifugation.

Procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Use disposable, individually wrapped, sterile plastic ware and sterile, disposable RNase-free pipettes, pipettes tips, and tubes.
- Wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin; change gloves frequently, particularly as the protocol progresses from crude extracts to more purified materials.

Abbreviated Protocol

1. Homogenization：1 ml Azol® NC RNA Isolation reagent up to 100 mg tissue or 10^7 cells.
2. DNA/protein precipitation：homogenate + 0.4 ml water, wait 5-15 min, 12,000 g x 15 min.
3. BAN purification (optional)：1 ml supernatant + 5 µl 4-bromoanisole, wait 3-4 min, 12,000 g x 10 min.
4. RNA precipitation：supernatant + 1 volume isopropanol, wait 15 min, 12,000 g x 10 min.
5. RNA washes：0.4 ml 75% ethanol, 4,000 g x 1-3 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

This protocol yields all classes of RNA in one fraction containing:
large nuclear RNA, rRNA, mRNA, small RNA and microRNA down to 10 bases.

Attention :

※ Reagents required but not supplied: ethanol, isopropanol, 4-bromoanisole (BAN, optional) and RNase-free water.

※ The isolation is performed at room temperature and centrifugate at 4 - 28°C.

HOMOGENIZATION.

A. Tissues

Add 1 mL of Azol® NC RNA isolation reagent 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 addition of Azol® NC RNA isolation reagent.

1. Remove culture medium and add at least 1 ml of the reagent per 3.5 cm culture dish (10 cm^2).
2. Pass the lysate through a pipette several times to ensure lysis.

Cells grown in suspension should be sedimented first and then lysed by the addition of Azol® NC RNA isolation reagent.

1. Add at least 1 ml of Azol® NC RNA isolation reagent per 10^7 cells and lyse cells by repeated pipetting.
2. Washing cells before the addition of Azol® NC RNA isolation reagent is not recommended as it may contribute to RNA degradation.

For cells grown in monolayer, use the amount of Azol® NC RNA isolation reagent based on the area of the culture dish and not on cell number.
An insufficient amount of Azol® NC RNA isolation reagent will result in DNA contamination of the isolated RNA.

PROTOCOL (continued)

DNA, PROTEIN AND POLYSACCHARIDE PRECIPITATION.

1. Add to the homogenate/lysate 0.4 ml of water (RNase free water) per 1 ml of NC RNA extraction reagent used for homogenization.
2. Shake the resulting mixture vigorously for 15 seconds and store for 5 - 15 minutes at room temperature (20–25°C) . Samples with 100 mg tissue/ml Azol® NC RNA Isolation reagent require a 15 minutes storage at room temperature.
3. Centrifuge sample at 12,000 g for 15 minutes. Following centrifugation, DNA, proteins and most polysaccharides form a semisolid pellet at the bottom of the tube.
4. The RNA remains soluble in the supernatant.
5. Transfer 1 ml of the supernatant (75% of total supernatant volume) to a new tube, leaving a layer of the supernatant above the DNA/protein pellet.

PRECIPITATION OF TOTAL RNA.

6. Precipitate RNA by mixing 1 ml of the supernatant with 1 ml of isopropanol.
7. Store samples for 10 minutes at room temperature (20–25°C) and centrifuge at 12,000 g for 10 minutes. In most cases, RNA precipitate forms a white pellet at the bottom of a tube.

RNA WASHES.

8. Wash the RNA by mixing the pellet twice with 75% ethanol (v/v).
9. Centrifuge the pellet at 4,000-8,000 g for 1-3 minutes. Remove the alcohol solution using a micropipette.

RNA SOLUBILIZATION.

10. Dissolve the RNA pellet, without drying, in RNase-free water and stored at -70°C.
11. 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.