MRE-N3200 200 ML

USER GUIDE:NC RNA extraction reagent

PRODUCT DESCRIPTION:

NC RNA extraction 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. NC RNA extraction reagent isolates pure and undegraded RNA that is ready for the following application without DNase treatment.

NC RNA extraction 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 NC RNA extraction 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 NC RNA extraction reagent procedure is performed at room temperature, including centrifugation.

STABILITY/STORAGE: NC RNA extraction reagent is stable, when storing at 4-30°C, for at least one year.

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.

PROTOCOL FOR ISOLATION OF TOTAL RNA

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

※ Reagents required but not supplied: ethanol, isopropanol, 4-bromoanisole (BAN, optional) and RNase-free water.
※ The isolation is performed at room temperature and centrifugated at 4-28°C.

1. HOMOGENIZATION.

A. Tissues.

Add 1 mL of NC RNA extraction 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 NC RNA extraction reagent. 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 the addition of NC RNA extraction reagent. Add at least 1 ml of NC RNA extraction reagent per 10^7 cells and lyse cells by repeated pipetting. Washing cells before the addition of NC RNA extraction reagent is not recommended as it may contribute to RNA degradation. For cells grown in monolayer, use the amount of NC RNA extraction reagent based on the area of the culture dish and not on cell number. An insufficient amount of NC RNA extraction reagent will result in DNA contamination of the isolated RNA.

2. DNA, PROTEIN AND POLYSACCHARIDE PRECIPITATION.

Add to the homogenate/lysate 0.4 ml of water per 1 ml of NC RNA extraction reagent used for homogenization. Shake the resulting mixture vigorously for 15 seconds and store for 5 - 15 minutes. Samples with 100 mg tissue/ml NC RNA extraction reagent require a 15 minutes storage at room temperature. 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. The RNA remains soluble in the supernatant. 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. Centrifugation at this and other steps of the protocol can be performed at 4-28°C.

3. PHASE SEPARATION (OPTIONAL STEP).

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 and/or extracellular material. Add 5 µl (0.5% of the supernatant volume) of 4-bromoanisole to 1ml of the transferred supernatant. Shake the resulting mixture for 15 seconds, store it for 3-5 minutes and centrifuge it at 12,000 g for 10 minutes at 4-25°C. After centrifugation, residual DNA, protein and polysaccharide precipitate accumulates in the organic phase at the bottom of a tube, while RNA remains soluble in the supernatant.

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 above to a new tube. Precipitate RNA by mixing 1 ml of the supernatant with 1 ml of isopropanol. Store samples for 10 minutes and centrifuge at 12,000 g for 10 minutes. In most cases, RNA precipitate forms a white pellet at the bottom of a tube.

5. RNA WASHES.

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

6. RNA SOLUBILIZATION.

Dissolve the RNA pellet, without drying, in RNase-free water and stored 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.

MRE-N3200 NC RNA Extraction Reagent

Isolation of Total RNA - Abbreviated Protocol

1. Homogenization - 1 ml NC RNA extraction 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.