RNA reagent

Cat No	Pack size
RN0100	100 ml
RN0200	200 ml

RNA REAGENT

stability of 12 months ,we recommend **storage at 2 to 8℃** for optimal performance .isolation of a variety of RNA species has an A260/A280 ratio 1.8 when diluted into TE.

Description

RNA REAGENT is a ready-to-use reagent for the isolation of total RNA from cells and tissues plant. The reagent, is single-step RNA isolation method during sample homogenization or lysis. Addition of chloroform followed by centrifugation, separates an aqueous phase and an organic phase. After transfer of the aqueous phase, the RNA is recovered by precipitation with isopropyl alcohol. After removal of the aqueous phase, the DNA and proteins in the sample can be recovered by sequential precipitation. This reagent every 1ml well treat small quantities of tissue (50-100 mg) and cells ($5x10^6$)

Reagents required, but not supplied:

Chloroform Isopropyl alcohol
75% Ethanol (in DEPC-treated water)
RNase-free water or 0.5% SDS solution
[To prepare RNase-free water, draw water into RNase-free glass bottles. Add diethylpyrocarbonate (DEPC) to 0.01% (v/v). Let stand overnight and autoclave. The SDS solution must be prepared using DEPC-treated, autoclaved water.]

Protocol

1. Homogenization

a. *Tissues*

Homogenize tissue samples in 1 ml of RNA REAGENT per 50-100 mg of tissue. The sample volume should not exceed 10% of the volume of RNA REAGENT used for homogenization.

b. *Cells Grown in Monolayer*

Lyse cells directly in a culture dish by adding 1 ml of RNA REAGENT to a 10^6-10^7 cells, and pipette the cell lysate several times.

c. *Cells Grown in Suspension*

Pellet cells by centrifugation. Lyse cells in RNA REAGENT by repetitive pipetting. Use 1 ml of the reagent per $5x10^5$-10^6 of animal, plant or yeast cells, or per $1x10^7$ bacterial cells. Washing cells before addition of RNA REAGENT should be avoided the possibility of mRNA degradation.

Optional

An additional isolation step may be required for samples with high content of proteins, fat, polysaccharides and extracellular material. Following homogenization, remove insoluble material from the homogenate by centrifugation at 12,000xg for 10 minutes at 2 to 8℃. In samples from fat tissue, a top fat layer should be removed.

2. phase separation

Incubate the homogenized samples for 5 minutes at 15 to 30℃. Add 0.2 ml of chloroform per 1 ml of **RNA REAGENT**. Shake tubes vigorously for 15 seconds and centrifuge the samples not more than 12,000x *g* for 15 minutes at 2 to 8℃. Following centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The volume of the aqueous phase is about 60% of the volume of **RNA REAGENT** used for homogenization.

3. Precipitation

Transfer the aqueous phase to a fresh tube, and save the organic phase if isolation of DNA or protein is desired. Precipitate the RNA with 0.5 ml of isopropyl alcohol per 1ml of **RNA REAGENT** used for the initial homogenization. Incubate samples at 15 to 30℃ for 10 minutes and centrifuge at no more than 12,000x*g* for 10 minutes at 2 to 8℃. The RNA precipitate is often invisible.

4. Wash

Remove the supernatant. Wash the RNA pellet once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of **RNA REAGENT** used for the initial homogenization. Mix the sample by vortexing and centrifuge at no more than 7,500x*g* for 5 minutes at 2 to 8℃.

5. Redissolving the RNA

Briefly dry the RNA pellet (air-dry or vacuum-dry for 5-10minutes) Dissolve RNA in RNase-free water or 0.5% SDS solution by passing the solution a few times through a pipette tip, and incubating for 10 minutes at 55 to 60℃ (Avoid SDS when RNA will be used in subsequent enzymatic reactions.)

Troubleshooting Guide

1. **Low yield**

Homogenization or lysis of samples is not completely.
RNA pellet incompletely redissolved.

2 **A260/A280 ratio <1.65**

RNA sample was diluted in water or ETOH wash incompletely or contaminated with organic solvent. Sample homogenized and not stored at room temperature for 5 minutes.

3 **RNA degradation**

Tissues samples not immediately processed or frozen after removal from the animal.
Purified RNA were not stored at -60 to -70℃.
Redissolved solutions or tubes not RNase-free.

4 **Genomic DNA contamination**

Samples contained organic solvents, strong buffers, or alkaline solution.
Tissues samples is larger than 100mg or cell is more than $5x\ 10^6$.

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