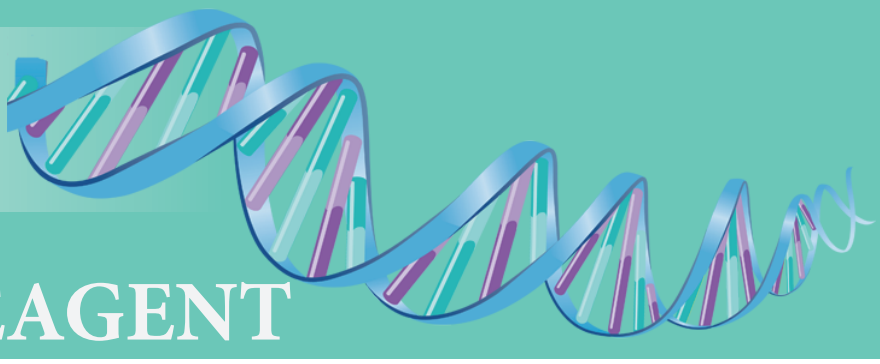
MORREBIO
The expert of life science



RNA Isolation REAGENT

Ordering information

Cat.	Quantity
RI200	200 ml

Stablity/ Storage

RNA Isolation REAGENT is stability of 12 months, we recommend storage at 2 to 8°C for optimal performance. Isolation of a variety of RNA species has an A260/A280 ratio 1.8 when diluted into TE.

Description

RNA Isolation 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 (5X 10^6)

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 diethyl pyrocarbonate (DEPC) to 0.01% (v/v). Let stand overnight and autoclave. The SDS solution must be prepared using DEPC-treated, autoclaved water.]

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Protocol

1. Homogenization

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

b. Cells Grown in Monolayer
Lyse cells directly in a culture dish by adding 1 ml of RNA Isolation REAGENT to a 106 - 107 cells, and pipette the cell lysate several times.

c. Cells Grown in Suspension
Pellet cells by centrifugation. Lyse cells in RNA Isolation REAGENT by repetitive pipetting. Use 1 ml of the reagent per 5 × 105 - 106 of animal, plant or yeast cells, or per 1 × 107 bacterial cells. Washing cells before addition of RNA Isolation 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 12,000 xg for 10 minutes at 2 to 8°C. 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°C. Add 0.2 ml of chloroform per 1 ml of RNA Isolation REAGENT. Shake tubes vigorously for 15 seconds and incubate them at 15 to 30°C for 2 to 3 minutes. Centrifuge the samples at no more than 12,000 xg for 15 minutes at 2 to 8°C. 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 Isolation REAGENT used for homogenization.

For Research Use Only.

About MORREBIO
Find more on https://www.light-biotech.com/

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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 Isolation REAGENT used for the initial homogenization. Incubate samples at 15 to 30°C for 10 minutes and centrifuge at no more than 12,000 xg for 10 minutes at 2 to 8°C. 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 Isolation REAGENT used for the initial homogenization. Mix the sample by vortexing and centrifuge at no more than 7,500 xg for 5 minutes at 2 to 8°C.

5. Redissolving the RNA
Briefly dry the RNA pellet (air-dry or vacuum-dry for 5- 10 minutes) 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°C (Avoid SDS when RNA will be used in subsequent enzymatic reactions.)

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