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Product Information

RNAzol® RT
For processing total and small RNA from human, animal, plant, bacterial, and viral samples

Catalog Number **R4533**
Store at room temperature.

TECHNICAL BULLETIN

Product Description

RNAzol RT is a quick and convenient reagent for use in the single-step isolation of total and small RNA from biological samples of human, animal, plant, yeast, bacterial, and viral origin. A convenient single-step liquid phase separation results in the isolation of RNA from DNA, protein, polysaccharides, and other molecules. RNAzol RT can be used to isolate separate fractions of mRNA and micro RNA or to isolate total RNA, containing all classes of RNA in a single fraction.

This product, a mixture of guanidine thiocyanate and phenol in a monophase solution, effectively dissolves DNA, RNA, and protein on homogenization or lysis of tissue sample. The addition of water to the mixture allows for the precipitation of DNA, proteins, polysaccharides and other molecules, which can be removed by centrifugation. RNA can then be isolated from the supernatant by alcohol precipitation, washing and solubilization. Chloroform-induced phase separation is not necessary. One mL of RNAzol RT is sufficient to isolate RNA from up to 100 mg of tissue, 1×10^7 cells, or 10 cm^2 of culture dish surface for cells grown in monolayer.

This is one of the most effective methods for isolating total and small RNA and can be completed at room temperature in less than 1 hour starting with fresh tissue or cells. The protocol for isolation of mRNA and micro RNA yields two fractions – an mRNA-containing fraction consisting of RNA of >200 bases and a micro RNA-containing fraction consisting of RNA of <200 bases. Isolation of total RNA is very effective for isolating RNA molecules of all types: large nuclear RNA, rRNA, mRNA, small RNA and micro RNA. The resulting RNA is intact with little or no contaminating DNA and protein that can be used for Northern blots, RNase protection assay, microarrays, polymerase chain reaction (PCR), and other molecular biology applications.

Reagents Required but Not Provided

mRNA and micro RNA Isolation:
- RNase-free water
- 75% Ethanol
- 70% Isopropanol
- 100% Isopropanol

Total RNA Isolation:
- RNase-free water
- 75% Ethanol
- 100% Isopropanol
- 4-bromoanisole (BAN, optional)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the product at room temperature.

Procedures

Sample Preparation

1A. Tissue:
Homogenize tissue samples in RNAzol RT (1 mL per up to 100 mg of tissue) using a Polytron® or other appropriate homogenizer.
Note: If the tissue has high DNA content (e.g. spleen tissue), use 1mL of reagent per 50 mg of tissue.

1B. Monolayer cells:
Lyse cells directly on the culture dish. After removing culture medium, use at least 1 mL of RNAzol RT per 10 cm^2 of glass culture plate surface area. After addition of the reagent, the cell lysate should be passed several times through a pipette to form a homogenous lysate.

Note: Washing cells prior to addition of RNAzol RT is not recommended, as this could contribute to RNA degradation.

1C. Suspension cells:
Isolate cells by centrifugation and then lyse in RNAzol RT by repeated pipetting. One mL of the reagent is sufficient to lyse 1 × 10^7 cells.

1D. Liquid samples:
Lyse using 1mL RNAzol RT per 0.4 mL of sample. For small volume samples, supplement the sample with RNase-free water to reach a sample volume of 0.4 mL prior to addition of 1mL RNAzol RT.

Notes:
a. If samples have a high content of fat, an additional step may be needed. After homogenization, centrifuge the homogenate at 12,000 × *g* for 5 minutes at 4-10 °C. There will be a layer of fatty material on the surface of the aqueous phase that should be removed. The supernatant contains RNA. Transfer the clear supernatant to a fresh tube and proceed with step 2.
b. After the cells have been homogenized or lysed in RNAzol RT, samples can be stored overnight at 4 °C or for at least one year at –20 °C.

2. DNA, protein, and polysaccharide precipitation:
Add 0.4 mL of RNase-free water per mL of RNAzol RT used for homogenization. Cover the sample tightly, shake vigorously for 15 seconds, and allow to stand for 5–15 minutes at room temperature. Centrifuge the resulting mixture at 12,000 × *g* for 15 minutes at 4-~~28~~ °C. Centrifugation separates the mixture into a semisolid pellet (containing DNA, proteins and polysaccharides) and an upper supernatant (containing RNA). Transfer supernatant to a new tube, leaving a layer of the supernatant above the DNA/protein pellet.

Notes:
a. Samples homogenized with 1mL RNAzol RT per 100 mg tissue require a 15 minute standing time.
b. Up to 85% of the supernatant can be collected for isolation of RNA.
c. Prior to total RNA isolation, an optional phase separation can be performed using 4-bromoanisole. Add 0.5% of the supernatant volume of 4-bromoanisole to the transferred supernatant and shake for 15 seconds to mix. Store at room temperature for 3-5 minutes then centrifuge at 12,000 × *g* for 10 minutes at 4-25 °C. Transfer the supernatant, which contains the RNA, to a new tube.

<u>mRNA and micro RNA Isolation</u>

1. Transfer the supernatant from previous step to a fresh tube and add 0.4 mL of 75% ethanol (v/v) to precipitate mRNA. Allow the sample to stand for 10 minutes at room temperature. Centrifuge at 12,000 × *g* for 8 minutes at 4-28 °C. The mRNA precipitate will form a white pellet on the side and bottom of the tube. Transfer supernatant to a new tube, leaving a layer of the supernatant above the mRNA pellet.

Notes:
a. Up to 85% of the supernatant can be collected for isolation of micro RNA.
b. This fraction contains mRNA and other RNA >200 bases, including large nuclear RNA and ribosomal RNA.
c. The micro-RNA containing supernatant can be stored at –20 °C for one year.

2. To the supernatant from Step 1, add 0.8 volume of 100% isopropanol. Allow sample to stand for 30 minutes at 4 °C. Centrifuge at 12,000 × *g* for 15 minutes at 4-28 °C. The micro RNA precipitate will form a white pellet on the bottom of the tube.

Note: This fraction contains micro RNA and other RNA <200 bases, including small ribosomal RNA and tRNA.

3. Wash RNA pellets from Steps 1 and 2 <u>twice</u> with 0.4-0.6 mL 75% ethanol (v/v) and 70% isopropanol (v/v), respectively, per 1mL of supernatant used for precipitation. Centrifuge at 4,000-8,000 × *g* for 1-3 minutes at room temperature. Remove alcohol solution with a micropipette.

4. Solubilize the RNA pellets, without drying, in RNase-free water at a concentration of 1-2 µg/µL for mRNA and 0.1 µg/µL for micro RNA. Vortex the samples at room temperature for 2-5 minutes.
   Notes:
   a. Drying the RNA pellet can decrease solubility and is not recommended.
   b. Final preparation of mRNA is free of DNA and proteins. It should have a A_{260}/A_{280} ratio of 1.8-2.1 and a A_{260}/A_{230} ratio of 1.8 to 2.3.
   c. Typical mRNA yields from tissues (µg RNA/mg tissue): liver, 5-7 µg; kidney, spleen, 3-4 µg; skeletal muscle, brain, lung, 0.5-1.5 µg; placenta, 1-3 µg.
   d. Typical mRNA yields from cultured cells (µg RNA/10^6 cells): epithelial cells, 5-8 µg; fibroblasts, 3-5 µg.
   e. Final preparation of micro RNA is free of DNA and proteins. It should have a A_{260}/A_{280} ratio of 1.6-1.7 and a A_{260}/A_{230} ratio of about 1.5.

Total RNA Isolation

Add 1-2 ul of LPA (GenElute-LPA, #56575) to each sample for better precipitation.

1. Transfer the supernatant from the preparation step to a fresh tube and add an equal volume of 100% isopropanol to precipitate RNA. Allow to stand for 10 minutes then centrifuge at ~~12,000 × *g* for 10 minutes at room temperature.~~ The RNA precipitate will form a white pellet on the bottom of the tube.

15,000 xg, 4C, 15 min

1 ml of 80% ethanol

2. Wash RNA pellets from Steps 1 twice with ~~0.4-0.6 mL 75% ethanol (v/v)~~ per 1mL of supernatant used for precipitation. Centrifuge at ~~4,000-8,000 × *g* for 1-3 minutes at room temperature.~~ Remove alcohol solution with a micropipette.

max speed, 4C, 1 min

Air dry 5-10 min at RT.

3. Solubilize the RNA pellet, without drying, in RNase-free water at a concentration of 1-2 µg/mL for mRNA and 0.1 µg/µL for micro RNA. Vortex the samples at room temperature for 2-5 minutes.

Resuspend in 15-30 ul of nuclease-free water with 1:20 (v/v) Superase-In. *Or alternative RNase Inhibitors at 1 U/ul.

Notes:
   a. Drying the RNA pellet can decrease solubility and is not recommended.
   b. Final preparation of RNA is free of DNA and proteins. It should have a A_{260}/A_{280} ratio of 1.7-2.1 and a A_{260}/A_{230} ratio of 1.6 to 2.3.
   c. Typical RNA yields from tissues (µg RNA/mg tissue): liver, 6-8 µg; kidney, spleen, 3-4 µg; skeletal muscle, brain, lung, 0.5-1.5 µg; placenta, 1-3 µg.

Max 5 min regardless dryness of RNA pellet.

Make sure adequate amount of RNAzol and vortex mix thoroughly.

Troubleshooting Guide

1. RNA Isolation:
   A. Low yield may be due to:
      - incomplete homogenization or lysis of samples.
      - the final RNA pellet may not have been completely dissolved.
   B. If the A_{260}/A_{280} ratio is <1.6:
      - the amount of sample used for homogenization may have been too small.
      - the OD measurement may have been performed with acidic water.
      - the final RNA pellet may not have been completely dissolved.
      - the sample may have been contaminated with protein or polysaccharide.
   C. If there is degradation of the RNA:
      - the tissues may not have been immediately processed or frozen after removing from the animal.
      - the samples used for isolation or the isolated RNA preparations may have been stored at –20 °C instead of –70 °C as specified in the procedure.
      - cells may have been dispersed by trypsin digestion.
      - aqueous solutions or tubes used for procedure may not have been RNAse-free.
   D. If there is DNA contamination:
      - the volume of reagent used for the sample homogenization may have been too small.
      - samples used for the isolation may have contained organic solvents (ethanol, DMSO), strong buffers or alkaline solution.

References

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JJJ,PHC 12/12-1