PRODUCT: **TRI REAGENT® RT - RNA / DNA / PROTEIN ISOLATION REAGENT** August 2023
Cat. No: RT 111
Storage: Store at room temperature.

PRODUCT DESCRIPTION

TRI Reagent® RT is an upgraded, patented (US patent 7,794,932) reagent for the single-step method of RNA isolation. Unlike the previous single-step methods, TRI Reagent® RT isolates RNA that is free of DNA contamination, as tested by 35-cycle PCR. Thus, no DNase treatment is necessary to use the isolated RNA in RT-PCR.

TRI Reagent® RT provides a reliable and cost-effective method of RNA isolation. TRI Reagent® RT combines phenol and guanidine thiocyanate in a mono-phase solution to facilitate effective inhibition of RNase activity. A biological sample is homogenized or lysed in TRI Reagent® RT and the homogenate is separated into aqueous and organic phases by bromoanisole addition and centrifugation. After phase separation, RNA remains in the aqueous phase while DNA and proteins are sequestered into the interphase and organic phase. RNA is precipitated from the aqueous phase by addition of isopropanol, washed with ethanol and solubilized. DNA and proteins can be isolated from the interphase and organic phase.

TRI Reagent® RT isolates RNA from a wide variety of samples of human, animal, plant, yeast, bacterial and viral origin. RNA isolation is completed in less than 1 hour.

STABILITY: TRI REAGENT® RT is stable at 25 C for at least two years from the date of purchase.

SPECIAL HANDLING PRECAUTIONS

TRI Reagent RT contains a poison (phenol) and an irritant (guanidine thiocyanate). Causes burns. CAN BE FATAL. When working with TRI Reagent RT **use gloves and eye protection** (shield, safety goggles). Do not get on skin or clothing. Avoid breathing vapor. Read the warning note on the bottle and MSDS. **In case of contact:** Immediately flush eyes or skin with a large amount of water for at least 15 min and seek immediate medical attention.

PROTOCOL FOR ISOLATION OF RNA

The TRI Reagent RT procedure can be completed in 1 h and the isolated RNA is free of DNA contamination, as tested by 35-cycle PCR. TRI Reagent RT isolates high quality RNA from diverse biological material, including animal and plant tissues rich in polysaccharides and proteoglycans. The isolated RNA can be used for RT-PCR, northern analysis, dot blot hybridization, poly A^+ selection, in vitro translation, RNase protection assay and molecular cloning.

Reagents required but not supplied: 4-bromoanisole (BAN, Cat. No. BN 191), isopropanol and ethanol. We recommend the use of disposable polypropylene tubes provided by Molecular Research Center, Inc. Tubes from other suppliers should be tested to ensure integrity during centrifugation at 12,000 g with TRI Reagent RT.

The isolation is carried out at room temperature unless stated otherwise. The protocol includes the following steps:

---

1. HOMOGENIZATION - 1 ml TRI Reagent RT + 50 - 100 mg tissue, 5 - 10 x 10^6 cells or 10 cm^2 of culture plate.
2. PHASE SEPARATION - homogenate + 50 µl bromoanisole, at 4 C.
3. RNA PRECIPITATION - 0.5 ml aqueous phase + 0.5 ml isopropanol.
4. RNA WASH - 1 ml 75% ethanol.
5. RNA SOLUBILIZATION - water or FORMAzol®.

---

1. HOMOGENIZATION

A. TISSUES. Homogenize tissue samples with TRI Reagent RT in a glass-Teflon or Polytron homogenizer. Use 50 - 100 mg tissue per 1 ml TRI Reagent RT. **Sample volume should not exceed 10% of the volume of TRI Reagent RT** used for homogenization. Tissues with high DNA content, such as spleen or liver, should be processed using ≤ 50 mg tissue / 1 ml of TRI Reagent RT. For the optimal processing of muscle samples use 100 mg tissue / 1 ml TRI Reagent RT.

B. CELLS. Cells grown in monolayer should be lysed directly in a culture dish. Pour off media, add TRI Reagent RT and pass the cell lysate several times through a pipette. Use 1 ml of TRI Reagent RT per 10 cm^2 of culture dish area. See Note 3.
Cells grown in suspension should be sedimented first and then lysed in TRI Reagent RT by repetitive pipetting. Use 1.0 ml of the reagent per 5 - 10 x 10^6 animal, plant or yeast cells or per 10^7 bacterial cells.
Avoid washing cells before the addition of TRI Reagent RT as this may contribute to mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

2. PHASE SEPARATION

Supplement homogenate with 50µl of bromoanisole per 1 ml of TRI Reagent RT used for homogenization. Cover the samples tightly and shake vigorously for 15 seconds. Centrifuge the resulting mixture at 12,000 g for 15 minutes at 4 C. Following centrifugation, the mixture separates into a lower, red phenol phase, the interphase, and the upper aqueous phase. RNA remains in the aqueous phase whereas DNA and proteins are in the interphase and organic phase.

It is important to separate phases in the cold (4 - 10 C). Centrifugation performed at elevated temperatures may sequester DNA into the aqueous phase. The use of bromoanisole for phase separation improves the quality of isolated RNA and eliminates toxic chloroform and bromochloropropane from the isolation protocol.

3. RNA PRECIPITATION

The resulting aqueous phase constitutes about 70% of the volume of TRI Reagent RT used for homogenization plus sample volume (mg = µl). Collect 0.5 ml of the aqueous phase and transfer it to a fresh tube. During the collection, leave the remaining aqueous phase as an undisturbed layer to avoid collection of DNA from the interphase. Save the interphase and organic phase at 4 C or -20 C for subsequent isolation of DNA and proteins.

Precipitate RNA by mixing the aqueous phase with 0.5 ml of isopropanol. Store samples at room temperature for 5 - 10 minutes and centrifuge at 4,000 - 12,000 g for 5 minutes at 4 - 25 C. RNA precipitate (often invisible before centrifugation) forms a gel-like or white pellet on the side and bottom of the tube.

When isolating RNA from sources rich in polysaccharides and proteoglycans, perform the modified precipitation described in the Troubleshooting guide (last page).

4. RNA WASH

Remove the supernatant and wash the RNA pellet with 1 ml of 75% ethanol by vortexing and subsequent centrifugation at 4,000 - 6,000 g for 5 minutes at 4 - 25 C. If the RNA pellet accumulates on the side of the tube or has a tendency to float, sediment the pellet at 12,000 g. *The ethanol wash can be repeated to improve 260/280 ratio of the isolated RNA.*

5. RNA SOLUBILIZATION

Remove the ethanol wash and add water to the RNA pellet. Avoid drying the pellet as this will decrease its solubility. Hydrate the samples in water for 5 - 10 minutes at room temperature, or if necessary, at 55 - 60 C. Dissolve RNA by vortexing or passing the solution through a pipette tip few times. Typically, amount of water used for solubilization should be sufficient to obtain RNA concentration ranging 0.5 - 2 µg/µl.

Water used for the RNA solubilization should be RNase-free. Due to the higher purity, it is easier to dissolve RNA isolated by TRI Reagent RT as compared to previous single-step methods.

Alternatively, dissolve RNA in FORMAzol® (MRC Cat. No. FO 121). FORMAzol is a convenient medium to store RNA samples for a few days at room temperature or for long term storage at -20 C. It protects RNA from degradation due to accidental contamination during storage and handling of samples. Before use in RT-PCR, RNA samples dissolved in FORMAzol should be precipitated with ethanol or diluted at least 50 times in an RT mix.

6. RESULTS

The Bioanalyzer test or agarose gel electrophoresis of the isolated RNA shows predominant bands of small (~ 2 kb) and large (~ 5 kb) ribosomal RNA, low molecular weight (0.1 - 0.3 kb) RNA, and in some preparations discrete bands of high molecular weight (7 - 15 kb) RNA. The RNA Integrity Number (RIN) of the isolated RNA is > 8.5.

The isolated RNA is free of DNA and proteins and has a 260/280 ratio 1.6 - 1.9. For optimal spectrophotometric measurements, RNA aliquots should be diluted with water or buffer with a pH > 7.5. Distilled water with a pH < 7.0 falsely decreases the 260/280 ratio and impedes the detection of protein contamination in RNA samples (7).

Expected Yield: A) tissues (µg RNA/mg tissue): liver, spleen, 6 - 10 µg; kidney, 3 - 4 µg; skeletal muscles, brain, 1 - 1.5 µg; placenta, 1 - 4 µg; B) cultured cells (µg RNA/10^6 cells): epithelial cells, 8 - 15 µg; fibroblasts, 5 - 7 µg.

NOTES

1. To facilitate isolation of RNA from small samples (< 10^6 cells or < 10 mg tissue) perform homogenization or lysis in 0.8 ml of TRI Reagent RT supplemented with 2 - 3 µl of Polyacryl Carrier™ (Cat. No. PC 152). Next, add bromoanisole and proceed with the phase separation and other steps of isolation as described above.
2. After homogenization and before bromoanisole addition, samples can be stored at -20 C for at least one month. The RNA precipitate (Step 4, RNA Wash) can be stored in 75% ethanol at 4 C for at least one week or at -20 C for at least one year.
3. **For cells grown in monolayer, use the amount of TRI Reagent RT is based on the area of a culture dish and not on cell number.** Using an insufficient amount of TRI Reagent RT may result in DNA contamination of the isolated RNA.
4. Hands and dust may be a major source of the RNase contamination. Use gloves and keep tubes closed throughout the procedure.

5. An additional centrifugation step may be required for samples with a high content of proteins, fat, polysaccharides or extracellular material such as muscles, fat tissue and tuberous parts of plants. Following homogenization, remove insoluble material from the homogenate by centrifugation at 12,000 g for 10 minutes at 4 C. The resulting pellet contains extracellular membranes, polysaccharides and high molecular weight DNA while the supernatant contains RNA. In samples from fat tissue, an excess of fat collects as a top layer which should be removed. Transfer the clear supernatant to a fresh tube and proceed with the phase separation and RNA isolation as described above.
6. Whole blood and serum should be processed with RNAzol® BD (RB 192).

TROUBLESHOOTING GUIDE

RNA ISOLATION

Low yield. a) incomplete homogenization or lysis of samples, b) incomplete solubilization of the final RNA pellet.

260/280 ratio < 1.6. a) too small volume of the reagent used for sample homogenization, b) acidic water was used for the spectrophotometric measurement, c) contamination of the aqueous phase with phenol phase, d) incomplete solubilization of the final RNA pellet.

RNA degradation. a) tissues were not immediately processed or frozen after removing from animal, b) samples used for isolation, or the isolated RNA preparations were stored at -20 C instead of -70 C, c) cells were dispersed by trypsin digestion, d) aqueous solutions or tubes used for solubilization of RNA were not RNase-free, e) formaldehyde used for agarose-gel electrophoresis had a pH below 3.5.

DNA contamination. a) too small volume of the reagent was used for homogenization, b) samples used for the isolation contained organic solvents, strong buffers or alkaline solution, c) phase separation was performed at temperatures above 10 C.

Proteoglycan and polysaccharide contamination. The following modification of RNA precipitation (Step 3) removes these contaminating compounds from the isolated RNA (6). Add to the aqueous phase 0.25 ml of isopropanol followed by 0.25 ml of a high salt precipitation solution (0.8 M sodium citrate and 1.2 M NaCl) per 1 ml of TRI Reagent RT used for the homogenization. Mix the solution, store for 5 - 10 minutes at room temperature, and centrifuge at 12,000 g for 8 minutes at 4 - 25 C. Wash the resulting RNA pellet as described in Step 4 of the protocol. The modified precipitation effectively precipitates RNA while maintaining polysaccharides and proteoglycans in a soluble form. Also, low molecular weight RNA (>300 b) remains partially soluble. To isolate pure RNA from plant material containing a very high level of polysaccharides, the modified precipitation should be combined with an additional centrifugation of the initial homogenate described in Note 5 in the RNA Isolation protocol.

REFERENCES

1. Chomczynski P and Sacchi N (1987) Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem,162, 156-159.
2. Chomczynski P (1993) A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques,15, 532-537.
3. Mackey K and Chomczynski P (1996) Long-term stability of RNA isolation reagents. J NIH Res., 8,72.
4. Chomczynski P and Mackey K (1995) Substitution of chloroform with bromochloropropane in the single-step method of RNA isolation. Anal Biochem, 225, 163-164.
5. Ausubel F M, Brent R, Kingston R E, Moore D D, Seidman J G, Smith J A and Struhl K (1990) Appendix 1, in Current Protocols in Molecular Biology, vol 2, p. A.1.5, John Wiley and Sons, Inc., New York, NY.
6. Chomczynski P and Mackey K (1995) Modification of the TRI REAGENT RT™ procedure for isolation of RNA from polysaccharide - and proteoglycan - rich sources. Biotechniques,19, 942-945.
7. Wilfinger W, Mackey K and Chomczynski P (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques, 22, 474-481.
8. Wu, LC (1997) Isolation and Long-Term Storage of Proteins from Tissues and Cells Using TRIzol Reagent. FOCUS, 17, 98-100.
9. Banerjee, S, Smallwood A, Chambers AE and Nicolaides K. (2003) Quantitative Recovery of Immunoreactive Proteins from Clinical Samples Following RNA and DNA Isolation. Biotechniques, 35, 450-456.

Use of TRI Reagent® RT can be cited by referring to this brochure: TRI Reagent® RT - RNA, DNA, protein isolation reagent. Manufacturer's protocol (2008-2023), Molecular Research Center, Inc. Cincinnati, OH.