

TRIZOL® Reagent
Total RNA Isolation Reagent

WARNING: Toxic in contact with skin and if swallowed. Causes burns. After contact with skin, wash immediately with plenty of detergent and water. If you feel unwell, seek medical advice (show label where possible). Phenol (108-95-2) and Other Components (NJSRN 80100437-5000p).

Cat. No. 15596 100 mL
 200 mL

Storage Conditions: 2 to 8°C

Description

TRIZOL Reagent (U.S. Patent No. 5,346,994) is a ready-to-use reagent for the isolation of total RNA from cells and tissues. The reagent, a mono-phasic solution of phenol and guanidine isothiocyanate, is an improvement to the single-step RNA isolation method developed by Chomczynski and Sacchi¹. During sample homogenization or lysis, TRIZOL Reagent maintains the integrity of the RNA, while disrupting cells and dissolving cell components. Addition of chloroform followed by centrifugation, separates the solution into an aqueous phase and an organic phase. RNA remains exclusively in the aqueous 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. Precipitation with ethanol yields DNA from the interphase, and an additional precipitation with isopropyl alcohol yields proteins from the organic phase². Copurification of the DNA may be useful for normalizing RNA yields from sample to sample.

This technique performs well with small quantities of tissue (50-100 mg) and cells (5×10^6), and large quantities of tissue (≥ 1 g) and cells ($>10^7$), of human, animal, plant, or bacterial origin. The simplicity of the TRIZOL Reagent method allows simultaneous processing of a large number of samples. The entire procedure can be completed in one hour. Total RNA isolated by TRIZOL Reagent is free of protein and DNA contamination. It can be used for Northern blot analysis, dot blot hybridization, poly(A)⁺ selection, in vitro translation, RNase protection assay, and molecular cloning. For use in the polymerase chain reaction (PCR[®]), treatment of the isolated RNA with amplification grade DNase I (GIBCO BRL Cat. No. 18068) is recommended when the two primers lie within a single exon.

TRIZOL Reagent facilitates isolation of a variety of RNA species of large or small molecular size. For example, RNA isolated from rat liver, electrophoresed on an agarose gel, and stained with ethidium bromide, shows discrete bands of high molecular weight RNA between 7 kb and 15 kb in size, two predominant ribosomal RNA bands at ~5 kb (28S) and at ~2 kb (18S), and low molecular weight RNA between 0.1 and 0.3 kb (tRNA, 5S). The isolated RNA has an $A_{260/280}$ ratio of 1.6-1.8. The expected yield of RNA per mg of tissue is: liver and spleen, 6-10 µg; kidney, 3-4 µg; skeletal muscles and brain, 1-5 µg; placenta, 1-4 µg. The expected yield of RNA from 1×10^6 cultured cells: epithelial cells, 8-15 µg; fibroblasts, 5-7 µg.

Reagents required, but not supplied:

- Chloroform (without any additives, such as isoamyl alcohol)
- 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.]

Precautions for Preventing RNase Contamination:

RNases can be introduced accidentally into the RNA preparation at any point in the isolation procedure through improper technique. Because RNase activity is difficult to inhibit, it is essential to prevent its introduction. The following guidelines should be observed when working with RNA.

- Always wear disposable gloves. Skin often contains bacteria and molds that can contaminate an RNA preparation and be a source of RNases. Practice good microbiological technique to prevent microbial contamination.
- Use sterile, disposable plasticware and automatic pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipment. For example, a laboratory that is using RNA probes will likely be using RNase A or T1 to reduce background on filters, and any nondisposable items (such as automatic pipettes) can be rich sources of RNases.
- In the presence of TRIZOL Reagent, RNA is protected from RNase contamination. Downstream sample handling requires that nondisposable glassware or plasticware be RNase-free. Glass items can be baked at 150°C for 4 hours, and plastic items can be soaked for 10 minutes in 0.5 M NaOH, rinsed thoroughly with water, and autoclaved.

Other Precautions

- Use of disposable tubes made of clear polypropylene is recommended when working with less than 2-mL volumes of TRIZOL Reagent.
- For larger volumes, use glass (Correx) or polypropylene tubes, and test to be sure that the tubes can withstand 12,000 x g with TRIZOL Reagent and chloroform. Do not use tubes that leak or crack.
- Carefully equilibrate the weights of the tubes prior to centrifugation.
- Glass tubes must be sealed with parafilm topped with a layer of foil, and polypropylene tubes must be capped before centrifugation.

NOTES:

1. To facilitate isolation of RNA from small quantities of sample ($<10^6$ cells or <10 mg tissue) perform homogenization (or lysis) of samples in 0.8 mL of TRIZOL Reagent. Following homogenization, add chloroform and proceed with the phase separation, as described in step 2. Prior to precipitating the RNA with isopropanol, add 5-10 µg RNase-free (Molecular Biology Grade) glycogen (as carrier) to the aqueous phase. [The GLASSMAX™ RNA Microisolation Spin Cartridge System (GIBCO BRL Cat. No. 18385), is also suggested for use in these cases. The system is particularly suited for use with small quantities of cells ($\leq 10^6$) and tissues (≤ 20 mg), where the total RNA isolated is to be used in RT-PCR. No carrier is required.]
2. An additional isolation step may be required for samples with 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 x g for 10 minutes at 2-8°C. The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while the supernate contains RNA. In samples from fat tissue, an excess of fat collects as a top layer which should be removed. In each case, transfer the cleared homogenate solution to a fresh tube and proceed with chloroform addition and phase separation as described.

3. After homogenization and before addition of chloroform, samples can be stored at -60 to -70°C for at least one month. The RNA precipitate (step 4, RNA WASH) can be stored in 75% ethanol at 2 to 8°C for at least one week, or at least one year at -5 to -20°C.
4. Table-top centrifuges that can attain a maximum of 2,600 x g are suitable for use in these protocols if the centrifugation time is increased to 30-60 minutes in steps 2 and 3.

INSTRUCTIONS FOR RNA ISOLATION:

CAUTION: When working with TRIZOL Reagent use gloves and eye protection (shield, safety goggles). Avoid contact with skin or clothing. Use in a chemical fume hood. Avoid breathing vapor.

Note: Unless otherwise stated, the procedure is carried out at 15 to 30°C, and reagents at 15 to 30°C. See page 2 for reagents required but not supplied, and for precautions.

1. HOMOGENIZATION (see notes 1-3)

a. Tissues

Homogenize tissue samples in 1 mL of TRIZOL Reagent per 50-100 mg of tissue using a glass-Teflon® or power homogenizer (Polytron, or Tekmar's TISSUMIZER® or equivalent). The sample volume should not exceed 10% of the volume of TRIZOL Reagent used for homogenization.

b. Cells Grown in Monolayer

Lyse cells directly in a culture dish by adding 1 mL of TRIZOL Reagent to a 3.5 cm diameter dish, and passing the cell lysate several times through a pipette. The amount of TRIZOL Reagent added is based on the area of the culture dish (1 mL per 10 cm²) and not on the number of cells present. An insufficient amount of TRIZOL Reagent may result in contamination of the isolated RNA with DNA.

c. Cells Grown in Suspension

Pellet cells by centrifugation. Lyse cells in TRIZOL Reagent by repetitive pipetting. Use 1 mL of the reagent per $5-10 \times 10^6$ of animal, plant or yeast cells or per 1×10^7 bacterial cells. Washing cells before addition of TRIZOL Reagent should be avoided as this increases the possibility of mRNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

2. PHASE SEPARATION

Incubate the homogenized samples for 5 minutes at 15 to 30°C to permit the complete dissociation of nucleoprotein complexes. Add 0.2 mL of chloroform per 1 mL of TRIZOL Reagent. Cap sample tubes securely. Shake tubes vigorously by hand 15 seconds and incubate them at 15 to 30°C for 2 to 3 minutes. Centrifuge samples at no more than 12,000 x g for 15 minutes at 2 to 8°C. Following centrifugation, the mixture separates into a lower red, phenol-chloroform phase, 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 TRIZOL Reagent used for homogenization.

3. RNA 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 from the aqueous phase by mixing with isopropyl alcohol. Use 0.5 mL of isopropyl alcohol per 1 mL of TRIZOL Reagent used for the initial homogenization. Incubate samples at 15 to 30°C for 10 minutes and centrifuge at no more than 12,000 x g for 10 minutes at 2 to 8°C. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.

4. RNA WASH

Remove the supernate. Wash the RNA pellet once with 75% ethanol, adding at least 1 mL of 75% ethanol per 1 mL of TRIZOL Reagent used for the initial homogenization. Mix the sample by vortexing and centrifuge at no more than 7,500 g for 5 minutes at 2 to 8°C.

5. REDISSOLVING THE RNA

At the end of the procedure, briefly dry the RNA pellet (air-dry or vacuum-dry for 10 minutes). Do not dry the RNA by centrifugation under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility. Partially dissolved RNA samples have an $A_{260/280}$ ratio < 1.6 . Dissolve RNA in RNase-free water or 0.5% SDS solution by passing the solution a few times through pipette tip, and incubating for 10 minutes at 55 to 60°C.

INSTRUCTIONS FOR DNA ISOLATION:

After complete removal of the aqueous phase, as described in the RNA isolation protocol, the DNA in the interphase and phenol phase from the initial homogenate may be isolated. Following precipitation and a series of washes, the DNA is solubilized in 8 mM NaOH. Full recovery of DNA from tissues and culture cells permits the use of TRIZOL Reagent for the determination of the DNA content in analyzed samples³. Simultaneous extraction of genomic DNA allows for normalization of the results of Northern analysis per genome DNA instead of the more variable total RNA or tissue weight. (Depending on the source, the DNA pellet obtained may require additional purification (e.g., phenol extraction) prior to other applications.)

Reagents required, but not supplied:

- Ethanol
- 0.1 M Sodium citrate in 10% ethanol
- 75% Ethanol
- 8 mM NaOH

Unless otherwise stated, the procedure is carried out at 15 to 30°C.

1. DNA PRECIPITATION

Remove the remaining aqueous phase overlying the interphase, and precipitate the DNA from the interphase and organic phase with ethanol. Add 0.3 mL of 100% ethanol per 1 mL of TRIZOL Reagent used for the initial homogenization, and mix samples by inversion. Next, store the samples at 15 to 30°C for 2-3 minutes and sediment DNA by centrifugation at no more than 2,000 x g for 5 minutes at 2 to 8°C. Careful removal of the aqueous phase is critical for the quality of the isolated DNA.

2. DNA WASH

Remove the phenol-ethanol supernate, and if desired, save it for protein isolation. Wash the DNA pellet twice in a solution containing 0.1 M sodium citrate in 10% ethanol. Use 1 mL of the solution per 1 mL of TRIZOL Reagent used for the initial homogenization. At each wash, store the DNA pellet in the washing solution for 30 minutes at 15 to 30°C (with periodic mixing) and centrifuge at 2,000 x g for 5 minutes at 2 to 8°C. Following these two washes, suspend the DNA pellet in 75% ethanol (1.5-2 mL of 75% ethanol per 1 mL TRIZOL Reagent), store for 10-20 minutes at 15 to 30°C (with periodic mixing) and centrifuge at 2,000 x g for 5 minutes at 2 to 8°C.

An additional wash in 0.1 M sodium citrate-10% ethanol solution is required for large pellets, containing > 200 µg DNA or large amounts of a non-DNA material.

3. REDISSOLVING THE DNA

Briefly dry the DNA pellet for 5-10 minutes under vacuum and dissolve in 8 mM NaOH by slowly passing the pellet through a pipette. Add an adequate amount of 8 mM NaOH to approach a DNA concentration of 0.2-0.3 µg/µL. Typically, add 0.3-0.6 mL of 8 mM NaOH to the DNA isolated from 50-70 mg of tissue or 1 x 10⁷ cells. The use of a mild alkaline solution assures full solubilization of the DNA pellet. At this stage, however, the DNA preparations (especially from tissues) still contain insoluble gel-like material (fragments of membranes, etc.). Remove the insoluble material by centrifugation at 12,000 x g for 10 minutes. Transfer the supernate containing DNA to a new tube.

QUANTITATION AND EXPECTED YIELDS OF DNA

Take an aliquot of the DNA preparation solubilized in 8 mM NaOH, mix it with water and measure the A₂₆₀ of the resulting solution. Calculate the DNA content using the A₂₆₀ value for double-stranded DNA. One A₂₆₀ unit equals 50 µg of double-stranded DNA/mL. For calculation of cell number in analyzed samples, assume that the amount of DNA per 1 x 10⁶ diploid cells of human, rat, and mouse origin equals: 7.1 µg, 6.5 µg, and 5.8 µg, respectively. The expected yield of DNA per mg of tissue is: 3-4 µg from liver and kidney; and 2-3 µg from skeletal muscles, brain and placenta. The expected yield of DNA per 1 x 10⁶ cultured human, rat and mouse cells is 5-7 µg.

APPLICATIONS

Amplification of DNA by PCR

After redissolving the DNA in 8 mM NaOH, adjust the pH to 8.4 with 0.1 M HEPES (see table). Add 0.1 to 1.0 µg of the DNA sample to your PCR reaction mixture and perform the standard PCR protocol.

Restriction endonuclease reactions

Adjust the pH of the DNA solution to a required value using HEPES (see table). Alternatively, samples may be dialyzed against 1 mM EDTA, pH 7 to pH 8.0. Use 3-5 units of enzyme per microgram of DNA. Use the conditions recommended by the manufacturer for the particular enzyme, and allow the reaction to proceed for 3 to 24 h. In a typical assay, 80-90% of the DNA is digestible.

pH Adjustment of DNA Samples Dissolved in 8 mM NaOH

(For 1 mL of 8 mM NaOH use the following amounts of 0.1 M or 1 M HEPES, free acid.)

Final pH	0.1 M HEPES (µL)	Final pH	1 M HEPES (µL)
8.4	66	7.2	30
8.2	90	7.0	42
	115		
	135		
	180		

Notes:

- The phenol phase and interphase can be stored at 2 to 8°C overnight.
- Samples suspended in 75% ethanol can be stored at 2 to 8°C for months.
- Samples dissolved in 8 mM NaOH can be stored overnight at 2 to 8°C. For long-term storage, adjust the pH to 7-8, and adjust the EDTA concentration to 1 mM.

INSTRUCTIONS FOR PROTEIN ISOLATION:

Proteins are isolated from the phenol-ethanol supernate obtained after precipitation of DNA with ethanol (step 1, DNA PRECIPITATION). The resulting preparation can be analyzed for the presence of specific proteins by Western blotting².

Reagents required, but not supplied:

- Isopropyl alcohol
- 0.3 M Guanidine hydrochloride in 95% ethanol
- Ethanol
- 1% SDS

1. PROTEIN PRECIPITATION

Precipitate proteins from the phenol-ethanol supernate (approximate volume 0.8 mL per 1 mL of TRIZOL Reagent) with isopropyl alcohol. Add 1.5 mL of isopropanol per 1 mL of TRIZOL Reagent used for the initial homogenization. Store samples for 10 minutes at 15 to 30°C, and sediment the protein precipitate at 12,000 x g for 10 minutes at 2 to 8°C.

2. PROTEIN WASH

Remove the supernate and wash the protein pellet 3 times in a solution containing 0.3 M guanidine hydrochloride in 95% ethanol. Add 2 mL of wash solution per 1 mL of TRIZOL Reagent used for the initial homogenization. During each wash cycle, store the protein pellet in the wash solution for 20 minutes at 15 to 30°C and centrifuge at 7,500 x g for 5 minutes at 2 to 8°C. After the final wash, vortex the protein pellet in 2 mL of ethanol. Store the protein pellet in ethanol for 20 minutes at 15 to 30°C and centrifuge at 7,500 x g for 5 minutes at 2 to 8°C.

3. REDISSOLVING THE PROTEIN PELLETT

Vacuum dry the protein pellet for 5-10 minutes. Dissolve it in 1% SDS by pipetting. Complete dissolution of the protein pellet may require incubating the sample at 50°C. Sediment any insoluble material by centrifugation at 10,000 x g for 10 minutes at 2 to 8°C, and transfer the supernate to a fresh tube. The sample is ready for use in Western blotting or may be stored at -5 to -20°C for future use.

²

The protein pellet suspended in 0.3 M guanidine hydrochloride-95% ethanol or in ethanol can be stored for at least one month at 2 to 8°C, or for at least one year at -5 to -20°C.

- The following protocol is an alternative approach that allows for more efficient recovery of proteins. Dialyze the phenol-ethanol supernate against three changes of 0.1% SDS at 2 to 8°C. Centrifuge the dialyzed material at 10,000 x g for 10 minutes. Use the clear supernate for Western blotting.

- Proteins may be quantified by the Bradford method as long as the concentration SDS is low enough (<0.1%) so that it will not interfere. Methods that do not have detergent-interface problems, and that do not rely on A₂₆₀/A₂₈₀ measurements may be used (traces of phenol may cause overestimation of protein concentrations).

References:

- Chomczynski, P., and Sacchi, N. *Anal. Biochem.* 162, 156 (1987).
- Chomczynski, P. *Biotechniques* 15, 532 (1993).
- Ausubel, F.M., et al, eds. *Current Protocols in Molecular Biology*, Vol.2, Greene Publishing Assoc. and Wiley-Interscience, New York, p.A.1.5 (1990).
- Simms, D., Cizdziel, P.E., Chomczynski, P. *Focus* 15.4, 99 (1993).

TROUBLESHOOTING GUIDE

RNA ISOLATION

- Expected yields of RNA per mg of tissue or 1 x 10⁶ cultured cells
 - Liver and spleen, 6-10 µg
 - Kidney, 3-4 µg
 - Skeletal muscles and brain, 1-1.5 µg
 - Placenta, 1-4 µg
 - Epithelial cells, 8-15 µg
 - Fibroblasts, 5-7 µg
- Low yield
 - Incomplete homogenization or lysis of samples.
 - Final RNA pellet incompletely redissolved.
- A_{260/280} ratio <1.65
 - Sample homogenized in too small a reagent volume.
 - Following homogenization, samples were not stored at room temperature for 5 minutes.
 - The aqueous phase was contaminated with the phenol phase.
 - Incomplete dissolution of the final RNA pellet.
- RNA degradation
 - Tissues were not immediately processed or frozen after removal from the animal.
 - Samples used for isolation, or the isolated RNA preparations were stored at -5 to -20°C, instead of -60 to -70°C.
 - Cells were dispersed by trypsin digestion.
 - Aqueous solutions or tubes were not RNase-free.
 - Formaldehyde used for agarose-gel electrophoresis had a pH below 3.5.
- DNA contamination
 - Sample homogenized in too small a reagent volume.
 - Samples used for the isolation contained organic solvents (e.g., ethanol, DMSO), strong buffers, or alkaline solution.
- Proteoglycan and polysaccharide contamination
 - The following modification of the RNA precipitation (step 3) removes these contaminating compounds from the isolated RNA. Add to the aqueous phase 0.25 mL of isopropanol followed by 0.25 mL of a high salt precipitation solution (1.2 M sodium citrate and 0.8 M NaCl) per 1 mL of TRIZOL Reagent used for the homogenization. Mix the resulting solution, centrifuge and proceed with the isolation as described in the protocol. The modified precipitation effectively precipitates RNA while maintaining polysaccharides and proteoglycans in a soluble form. A combination of the modified precipitation with an additional centrifugation of the initial homogenate (note #5, RNA isolation protocol) is required to isolate pure RNA from plant material containing a very high level of polysaccharides.

DNA ISOLATION

- Expected yields of DNA per mg of tissue or 1 x 10⁶ cultured cells
 - Liver and kidney, 3-4 µg
 - Skeletal muscles, brain, and placenta 2-3 µg
 - Cultured human, rat, and mouse cells, 5-7 µg
 - Fibroblasts, 5-7 µg
- Low yield
 - Incomplete homogenization or lysis of samples.
 - Final DNA pellet incompletely redissolved.
- A_{260/280} ratio <1.70
 - Phenol was not sufficiently removed from the DNA preparation. Wash the DNA pellet an additional time with 0.1 M sodium citrate in 10% ethanol.
- DNA degradation
 - Tissues were not immediately processed or frozen after removal from the animal.
 - Samples used for isolation, or the isolated RNA preparations were stored at -5 to -20°C, instead of -60 to -70°C.
 - Samples were homogenized with a Polytron or other high speed homogenizer.
- RNA contamination
 - Incomplete removal of aqueous phase.
 - DNA pellet insufficiently washed with 0.1 M sodium citrate in 10% ethanol.
- Other applications
 - Prior to use in PCR amplification, adjust the pH to 8.4.
 - For digestion of the DNA with restriction endonucleases, adjust the pH to the desired value, use 3-5 units of enzyme per µg of DNA, and allow the reaction to go for 3-24 hours under optimal conditions for the particular enzyme.
 - Typically 80-90% of the DNA is digested.

PROTEIN ISOLATION

- Low yield
 - Incomplete homogenization or lysis of samples.
 - Final DNA pellet incompletely redissolved.
- Protein degradation
 - Tissues were not immediately processed or frozen after removing from the animal.
- Band deformation in PAGE
 - Protein pellet insufficiently washed.

*PCR is covered by a patent held by Hoffman LaRoche Corporation.

For questions, please call the Life Technologies TECH-LINESM.

For laboratory research use only.
CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.