

TRI Reagent[®] Solution

RNA / DNA / Protein Isolation Reagent

Part Number AM9738 100 mL



A. Product Description

TRI Reagent^{*} solution is a complete and ready-to-use reagent for the isolation of total RNA or the simultaneous isolation of RNA, DNA, and protein from diverse biological material, including samples of human, animal, plant, yeast, bacterial, and viral origin. This highly reliable technique performs well with samples larger than ~5 mg tissue or 5×10^5 cultured cells.

TRI Reagent solution combines phenol and guanidine thiocyanate in a monophasic solution to rapidly inhibit RNase activity. A biological sample is homogenized or lysed in TRI Reagent solution, and the homogenate is then separated into aqueous and organic phases by adding bromochloropropane (BCP) and centrifuging. RNA partitions to the aqueous phase, DNA to the interphase, and proteins to the organic phase. Next the RNA is precipitated from the aqueous phase with isopropanol, and finally it is washed with ethanol and solubilized.

High yield and high quality RNA

The TRI Reagent procedure for RNA isolation can be completed in about 1 hr. The isolated RNA can be used for any downstream procedure such as RT-PCR[†], amplification for array analysis, hybridization assays, or in vitro translation. RNA yield can vary considerably between samples. Yield is dependent on factors such as sample type, health of the organism, thoroughness of sample disruption, and sample handling. The RNA yield expectations listed below assume ideal conditions for factors that the user can control.

* TRI Reagent is a registered trademark of Molecular Research Center, Inc.

† There is no RNA isolation method that consistently yields RNA that is free of contaminating DNA at the level of RT-PCR detection. We recommend treating RNA samples with DNase prior to RT-PCR if removal of residual DNA is required.

Figure 1.

Homogenization



1. Homogenize tissue samples in 10–20 volumes TRI Reagent solution. Homogenize cultured cells in 1 mL TRI Reagent solution per $5\text{--}10 \times 10^6$ cells, or per 10 cm² culture dish area.
2. Incubate the homogenate for 5 min at room temp.
3. (Optional) Centrifuge at 12,000 xg for 10 min at 4°C and transfer the supernatant to a fresh tube.

RNA Extraction

DNA &
protein
isolation



4. Add 100 μL BCP per 1 mL of TRI Reagent solution, mix well, and incubate at room temp for 5–15 min.
5. Centrifuge at 12,000 x g for 10–15 min at 4°C, then transfer the aqueous phase to a fresh tube.

RNA Precipitation and Wash



6. Add 500 μL of isopropanol per 1 mL of TRI Reagent solution, vortex for 5–10 sec, and incubate at room temp for 5–10 min.
7. Centrifuge at 12,000 x g for 8 min at 4–25°C, and discard the supernatant.
8. Add 1 mL of 75% ethanol per 1 mL of TRI Reagent solution.
9. Centrifuge at 7,500 x g for 5 min, remove the ethanol, and briefly air dry the RNA pellet.

RNA Solubilization



10. Dissolve RNA in the buffer of your choice.

Tissue (mammalian)	RNA yield per mg tissue	Cultured cells	RNA yield per 10 ⁶ cells
Liver, spleen	6–10 µg	Epithelial cells	8–15 µg
Kidney	3–4 µg	Fibroblasts	5–7 µg
Skeletal muscle, brain	1–1.5 µg		
Placenta	1–4 µg		

Isolation of DNA and protein from TRI Reagent homogenates

DNA and proteins can be isolated sequentially from the interphase and organic phase using the protocol posted at the following web address: www.ambion.com/techlib/append/supp

B. TRI Reagent Solution Storage and Handling

Store TRI Reagent solution at 4°C.

The manufacturer of TRI Reagent has determined that it is stable at 25°C for at least two years from the date of purchase.

Special Handling Precautions

TRI Reagent solution contains a poison (phenol) and an irritant (guanidine thiocyanate). **Contact with TRI Reagent solution will cause burns and can be fatal.** Use gloves and other personal protection when working with TRI Reagent solution.

C. Materials Not Provided

For RNA isolation

- Nuclease-free Water
- Equipment for grinding/homogenizing solid tissue
- Appropriately sized RNase-free centrifuge tubes with secure closures, compatible with phenol/chloroform (polypropylene, or polyallomer), and capable of withstanding centrifugal forces of 12,000 x g
- Centrifuge capable of 12,000 x g
- 1-bromo-3-chloropropane (BCP; recommended, e.g., MRC, Cat #BP 151), or chloroform *without* added isoamyl alcohol
- 100% isopropanol, ACS grade or better
- 100% ethanol, ACS grade or better

For RNA analysis

- Spectrophotometer, e.g., the NanoDrop® 1000A Spectrophotometer
- Agilent® bioanalyzer 2100, or reagents and apparatus for preparation and electrophoresis of agarose gels

D. RNA Isolation Procedure

Reagent preparation

Prepare 75% ethanol by mixing 250 μL nuclease-free water with 750 μL 100% ethanol per mL of TRI Reagent solution to be used. Include 10% overage to ensure a sufficient volume.

Procedure notes



IMPORTANT

Two major sources of RNase contamination are fingers and dust. Wear gloves at all times and keep samples covered.

- Unless stated otherwise, conduct the procedure at room temperature.
- An additional step may be required for samples that have a high content of protein, fat, polysaccharide, or extracellular material, such as muscle, fat tissue, or tuberous parts of plants. See step 3 on page 6.
- Ambion recommends using BCP in step 4 because it is less toxic than chloroform and its use reduces the possibility of contaminating RNA with DNA. Chloroform can be used for phase separation, but it should not contain isoamyl alcohol or any other additive.
- For samples smaller than 5 mg or 5×10^5 cells, please refer to Ambion's print or web catalog for information on kits suitable for RNA isolation from small samples: www.ambion.com/info/isokits

- 1. Homogenize tissue samples in 10–20 volumes TRI Reagent solution. Homogenize cultured cells in 1 mL TRI Reagent solution per $5\text{--}10 \times 10^6$ cells, or per 10 cm^2 culture dish area.**

For a detailed discussion of cell and tissue disruption for RNA isolation, see Ambion Technical Bulletin #183, available on our website: www.ambion.com/techlib/tb/tb_183.html.

Tissue samples

Handling fresh tissue: Immediately after dissection, inactivate RNases by any one of the following treatments:

- Homogenize in TRI Reagent solution immediately
- Freeze rapidly in liquid nitrogen (tissue pieces must be small enough to freeze in a few seconds)
- Submerge in a tissue storage buffer such as Ambion RNA/ater[®] Solution (P/N AM7020)

Handling frozen tissue: Weigh frozen tissue, break into pieces smaller than ~50 mg (keeping tissue completely frozen), and homogenize directly in TRI Reagent solution. Larger pieces of tissue, very hard or fibrous tissues, and tissues with a high RNase content must typically be ground to a powder in liquid nitrogen for maximum RNA yield.

Homogenizing tissue: Homogenize samples in 10–20 volumes TRI Reagent solution (e.g., 1 mL TRI Reagent solution per 50–100 mg tissue), using standard homogenization procedures.

The sample volume should not exceed 10% of the volume of TRI Reagent solution used for homogenization.

Cells grown in culture

Do not wash cells before lysing with TRI Reagent solution as this may contribute to mRNA degradation.

Cells grown in monolayer: Pour off media, add 1 mL of TRI Reagent solution per 10 cm² of culture dish area, and pass the cell lysate through a pipette several times to lyse cells and homogenize the sample. (Lyse directly in the culture dish.) Use the area of the culture dish, not the cell number, to determine the volume of TRI Reagent solution for lysis.

Cells grown in suspension: Pellet cells, then lyse in 1 mL of TRI Reagent solution per 5–10 × 10⁶ animal, plant, or yeast cells, or per 10⁷ bacterial cells, by repetitive pipetting or vortexing.

Thorough disruption of some yeast and bacterial cells may require the use of a homogenizer.

2. Incubate the homogenate for 5 min at room temp.

This 5 min room temperature incubation allows nucleoprotein complexes to completely dissociate.

Homogenized samples can be stored at -70°C for at least one month.

3. (Optional) Centrifuge at 12,000 xg for 10 min at 4°C and transfer the supernatant to a fresh tube.

This optional centrifugation is only required to remove insoluble material from homogenates that contain high amounts of protein, fat, polysaccharide, or extracellular material, such as muscle, fat tissue, and tuberous parts of plants. Centrifugation pellets extracellular membranes, polysaccharides, and high molecular weight DNA, leaving the RNA in the supernatant.

High molecular weight DNA can be recovered from the pellet by following the wash and solubilization steps of the DNA isolation protocol, available at: www.ambion.com/techlib/append/supp.

Fat tissue samples: Fat will form a layer on top of the aqueous phase; remove and discard this layer.

4. Add 100 μL BCP per 1 mL of TRI Reagent solution, mix well, and incubate at room temp for 5–15 min.

- Add 100 μL BCP per 1 mL of TRI Reagent solution used for homogenization. Alternatively, 200 μL of chloroform (*without* isoamyl alcohol) can be used in place of BCP.
- Cap the tubes tightly and shake vigorously for 15 sec.
- Incubate the mixture at room temperature for 5–15 min.

5. Centrifuge at 12,000 x g for 10–15 min at 4°C, then transfer the aqueous phase to a fresh tube.

- Centrifuge at 12,000 x g for 10–15 min at 4°C .



IMPORTANT

Centrifugation at temperatures $> 8^{\circ}\text{C}$ may cause some DNA to partition in the aqueous phase.

- Transfer the aqueous phase (colorless top layer) to a fresh tube.

RNA remains exclusively in the aqueous phase whereas DNA and protein are in the interphase and organic phase. The interphase and lower, red, organic phase can be stored at 4°C for subsequent isolation of DNA and protein. A protocol is posted at the following web address:

www.ambion.com/techlib/append/supp

6. Add 500 μ L of isopropanol per 1 mL of TRI Reagent solution, vortex for 5–10 sec, and incubate at room temp for 5–10 min.



NOTE

When isolating RNA from sources rich in polysaccharides and proteoglycans, we recommend the modified precipitation described in Troubleshooting on page

- Add 500 μ L of isopropanol per 1 mL of TRI Reagent solution used for sample homogenization.
- Vortex at moderate speed for 5–10 sec.
- Incubate the samples at room temp for 5–10 min.

7. Centrifuge at 12,000 x g for 8 min at 4–25°C, and discard the supernatant.

- Centrifuge at 12,000 x g for 8 min at 4–25°C.
- Carefully remove the supernatant without disturbing the pellet.
Precipitated RNA forms a gel-like or white pellet on the side and bottom of the tube.

8. Add 1 mL of 75% ethanol per 1 mL of TRI Reagent solution.

Add 1 mL of 75% ethanol per 1 mL TRI Reagent solution used for sample homogenization to each sample to wash the RNA pellets.

9. Centrifuge at 7,500 x g for 5 min, remove the ethanol, and briefly air dry the RNA pellet.

- Centrifuge at 7,500 x g for 5 min at 4–25°C.
If the precipitated RNA floats or does not form a compact pellet, repeat the centrifugation at 12,000 x g for 5 min to consolidate the pellet at the bottom of the tube.

- b. Remove the ethanol wash without disturbing the pellet.
- c. Remove all residual ethanol by centrifuging again briefly and removing the ethanol that collects with a fine tip pipette. Complete removal of ethanol is necessary for the RNA to perform well in downstream applications.
- d. Air dry the RNA pellet for 3–5 min.
Do not completely dry the RNA pellet as this will greatly decrease its solubility. Do not dry RNA by vacuum centrifugation.

10. Dissolve RNA in the buffer of your choice.

- a. Dissolve RNA in THE RNA Storage Solution (P/N AM7000, AM7001), Nuclease-free Water, or your choice of buffer‡ by passing the solution a few times through a pipette tip or by vigorous vortexing
The resuspension volume is determined by the size of the RNA pellet. 3–5 mm pellets typically require 300–500 μL . If necessary, increase the resuspension volume or incubate at 55–60°C to completely dissolve the pellet.
- b. Store at 4°C for immediate analysis. For long-term storage, store at –70°C or colder.

E. Assessing RNA Yield and Quality

RNA yield

Spectrophotometry: the concentration of an RNA solution can be determined by measuring its absorbance at 260 nm. Ambion scientists recommend using the NanoDrop 1000A Spectrophotometer (www.nanoambion.com) because it is extremely quick and easy to use; just measure 1.5 μL of the RNA sample directly.

‡ Ambion offers several products for RNA storage; these include:
THE RNA Storage Solution - P/N AM7000, AM7001
TE Buffer - P/N AM9860, AM9861
0.1mM EDTA - P/N AM9912
RNAsecure™ - P/N AM7005, AM7006, AM7010
Formamide - P/N AM9342, AM9344

Alternatively, the RNA concentration can be determined by diluting an aliquot of the preparation in TE (10 mM Tris-HCl pH 8, 1 mM EDTA) and reading the absorbance in a traditional spectrophotometer at 260 nm. To determine the RNA concentration in $\mu\text{g/mL}$, multiply the A_{260} by the dilution factor and the extinction coefficient ($1 A_{260} = 40 \mu\text{g RNA/mL}$).

$$A_{260} \times \text{dilution factor} \times 40 = \mu\text{g RNA/mL}$$

Fluorometry: if a fluorometer or a fluorescence microplate reader is available, Molecular Probes' RiboGreen[®] fluorescence-based assay for RNA quantitation is a convenient and sensitive way to measure RNA concentration. Follow the manufacturer's instructions for using RiboGreen.

RNA Quality

Microfluidic analysis: the Agilent 2100 bioanalyzer with Caliper's RNA LabChip[®] Kits provides better qualitative data than conventional gel analysis for characterizing RNA. When used with the Ambion RNA 6000 Ladder (P/N AM7152), this system can provide a fast and accurate size distribution profile of RNA samples. Follow the manufacturer's instructions for performing the assay.

Agarose gel electrophoresis: You can also assess the quality of your RNA sample by agarose gel electrophoresis. Protocols are available at: www.ambion.com/techlib/append/supp

Spectrophotometry: the A_{260}/A_{280} ratio of the RNA is an indication of its purity. The total RNA isolated with this procedure should have an A_{260}/A_{280} ratio of 1.8–2.2.

F. Troubleshooting

Low yield

The potential yield of RNA varies greatly depending on the source. Typical yields for certain tissues and cell cultures are provided in section [A. Product Description](#) starting on page 1.

Potential causes of low RNA yield:

- Inadequate sample homogenization or lysis
- Incomplete solubilization of the RNA pellet

- Introduction of RNase before or during the precipitation, or during the wash steps

Low A260/A280 ratio (<1.6)

- The volume of TRI Reagent used for sample homogenization was too low.
Use 1 mL TRI Reagent solution per 50–100 mg tissue, $5\text{--}10 \times 10^6$ cells, or 10 cm^2 culture dish area.
- Nucleoproteins were incompletely dissociated from the RNA.
Make sure that the homogenate is allowed to stand at room temperature during step [2](#) on page 6 for at least 5 min before further processing.
- Aqueous phase was contaminated with the phenol phase.
- RNA pellet was not completely dissolved.

RNA degradation

- Tissues were not immediately processed or frozen after removing from animal.
- Samples used for isolation, or the isolated RNA preparations, were not stored long-term at -70°C .
- Reagents or tubes used for solubilization of RNA were not RNase-free.

DNA contamination

- Not enough TRI Reagent solution was used for homogenization.
For cells grown in monolayer, use the area of the culture dish, not the cell number, to determine the appropriate volume of TRI Reagent needed.
- Samples contained organic solvents, strong buffers or alkaline solution.
- The chloroform used contained additives such as isoamyl alcohol (IAA) or other water soluble organic solvents (ethanol, DMSO).
- Phase separation (step [5](#) on page [6](#)) was performed at temperatures above 8°C .

Proteoglycan and polysaccharide contamination

The following modification of steps [6](#) and [7](#) on page 7 effectively precipitates RNA while leaving polysaccharides and proteoglycans in the supernatant.

- a. Transfer the aqueous phase from step [5](#) to a fresh tube. For each 1 mL of TRI Reagent solution used for the homogenization, add 250 μ L of isopropanol and 250 μ L of a high salt precipitation solution (e.g. 0.8 M sodium citrate and 1.2 M NaCl).
- b. Mix well, store for 5–10 min at room temperature, and centrifuge at 12,000 x g for 8 min at 4–25°C.
- c. Wash the resulting RNA pellet as described in steps [8](#) and [9](#) of the procedure.

When isolating RNA from plant material containing a very high level of polysaccharides, include the optional centrifugation described in step [3](#) on page 6 and use the modified RNA precipitation described above.

G. Appendix

Quality Control

TRI Reagent solution is tested functionally for RNA isolation. The integrity of the RNA obtained is evaluated on an Agilent 2100 bioanalyzer.

Obtaining the MSDS

To obtain Material Safety Data Sheets (MSDSs) for any chemical product supplied by Applied Biosystems or Ambion:

- At www.appliedbiosystems.com, select **Support**, then **MSDS**. Search by chemical name, product name, product part number, or MSDS part number. Right-click to print or download the MSDS of interest.
- At www.ambion.com, go to the web catalog page for the product of interest. Click **MSDS**, then right-click to print or download.
- E-mail (MSDS_Inquiry_CCRM@appliedbiosystems.com) or telephone (650-554-2756; USA) your request, specifying the catalog or part number(s) and the name of the prod-

uct(s). We will e-mail the associated MSDSs unless you request fax or postal delivery. Requests for postal delivery require 1–2 weeks for processing.

For the MSDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.



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