ambion | RNA
by life technologies

TRIzol® Reagent

Catalog Numbers	Quantity	Store at 2°C to 25°C
15596-026	100 mL	
15596-018	200 mL	

Description

TRIzol® Reagent is a ready-to-use reagent, designed to isolate high quality total RNA (as well as DNA and proteins) from cell and tissue samples of human, animal, plant, yeast, or bacterial origin, within one hour. TRIzol® Reagent is a monophasic solution of phenol, guanidine isothiocyanate, and other proprietary components which facilitate the isolation of a variety of RNA species of large or small molecular size. TRIzol® Reagent maintains the integrity of the RNA due to highly effective inhibition of RNase activity while disrupting cells and dissolving cell components during sample homogenization. TRIzol® Reagent allows for simultaneous processing of a large number of samples, and is an improvement to the single-step RNA isolation method developed by Chomcynski and Sacchi (Chomczynski & Sacchi, 1987).

TRIzol® Reagent allows the user to perform sequential precipitation of RNA, DNA, and proteins from a single sample (Chomczynski, 1993). After homogenizing the sample with TRIzol® Reagent, chloroform is added, and the homogenate is allowed to separate into a clear upper aqueous layer (containing RNA), an interphase, and a red lower organic layer (containing the DNA and proteins). RNA is precipitated from the aqueous layer with isopropanol. DNA is precipitated from the interphase/organic layer with ethanol. Protein is precipitated from the phenol-ethanol supernatant by isopropanol precipitation. The precipitated RNA, DNA, or protein is washed to remove impurities, and then resuspended for use in downstream applications.

- Isolated RNA can be used in RT-PCR, Northern Blot analysis, Dot Blot hybridization, poly(A)+ selection, *in vitro* translation, RNase protection assay, and molecular cloning.
- Isolated DNA can be used in PCR, Restriction Enzyme digestion, and Southern Blots.
- Isolated protein can be used for Western Blots, recovery of some enzymatic activity, and some immunoprecipitation.

Caution

TRIzol® Reagent contains phenol (toxic and corrosive) and guanidine isothiocyanate (an irritant), and may be a health hazard if not handled properly. Always work with TRIzol® Reagent in a fume hood, and always wear a lab coat, gloves and safety glasses. Contact your Environmental Heath and Safety (EH&S) department for proper work and disposal guidelines. Avoid direct contact with TRIzol® Reagent, because **contact to skin, eyes, or respiratory tract may cause chemical burns** to the exposed area. **If contact to skin or eyes occurs**, *immediately* wash the exposed area with copious amounts of water for 15 minutes and seek medical attention if necessary. If you inhale vapors, move to fresh air and seek medical attention if necessary. For more information, refer to the TRIzol® Reagent SDS (Safety Data Sheet), available from our web site at **www.lifetechnologies.com/support**.

Contents and Storage

TRIzol® Reagent is supplied in 100 mL (Cat. no. 15596-026) or 200 mL (Cat. no. 15596-018) volumes, and shipped at room temperature. Upon receipt, store TRIzol® Reagent at room temperature. TRIzol® Reagent is stable for 12 months when properly stored.

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Materials Needed

The following additional materials are needed, but not supplied for the isolation of RNA, DNA or proteins.

RNA Isolation	DNA Isolation	Protein Isolation
• Chloroform	• Chloroform	• Chloroform
• Isopropyl alcohol	• 100% ethanol	• Isopropyl alcohol
• 75% ethanol (in DEPC-treated water)	• 75% ethanol	• 100% ethanol
• RNase-free water or 0.5% SDS	• 0.1 M sodium citrate in 10% ethanol	• 0.3 M Guanidine hydrochloride in 95% ethanol
• Centrifuge and rotor capable of reaching up to 12,000 × *g*	• 8 mM NaOH	• 1% SDS
• Polypropylene microcentrifuge tubes	• Centrifuge and rotor capable of reaching up to 12,000 × *g*	• Centrifuge and rotor capable of reaching up to 12,000 × *g*
• Water bath or heat block (55–60°C)	• Polypropylene microcentrifuge tubes	• Polypropylene microcentrifuge tubes

Preparing Samples

Homogenizing samples

1. Determine your sample type, and perform homogenization at room temperature according to the table below. The sample volume should not exceed 10% of the volume of TRIzol® Reagent used for homogenization. Be sure to use the indicated amount of TRIzol® Reagent, because an insufficient volume can result in DNA contamination of isolated RNA.

Sample Type	Action
Tissues	1. Add 1 mL TRIzol® Reagent per 50–100 mg of tissue sample. 2. Homogenize sample using a glass-Teflon® or power homogenizer. **Note**: Process or freeze tissue samples *immediately* upon collection.
Adherent Cells (Monolayer)	1. Remove growth media from culture dish. 2. Add 1 mL TRIzol® Reagent directly to the cells in the culture dish per 10 cm^2 of culture dish surface area. **Note**: Add 1 mL TRIzol® Reagent for a 35 mm dish, 3 mL for a 60 mm dish, and 8 mL for a 100 mm dish. 3. Lyse the cells directly in the culture dish by pipetting the cells up and down several times.
Suspension Cells	1. Harvest cells by centrifugation and remove media. 2. Add 0.75 mL of TRIzol® Reagent per 0.25 mL of sample (5–10 × 10^6 cells from animal, plant or yeast origin, **or** 1 × 10^7 cells of bacterial origin). **Note**: Do not wash cells before addition of TRIzol® Reagent to avoid increased chance of mRNA degradation. 3. Lyse cells in sample by pipetting up and down several times. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

2. *(Optional)* When preparing samples with high content of fat, proteins, polysaccharides, or extracellular material (e.g., muscle, fat tissue, or tuberous plant material), an additional isolation step may be required to remove insoluble material from the samples.

   Note: **Do not** perform this additional isolation step if you are performing subsequent DNA isolation on your sample.

Sample Type	Notes
Tissue or cells with high content of fat, protein, polysaccharide, or extracellular material	1. Following homogenization, centrifuge your sample at 12,000 × *g* for 10 minutes at 4°C. **Note**: The resulting pellet contains ECM, polysaccharides, and high molecular weight DNA, while the supernatant contains the RNA. In high fat content samples, a layer of fat collects above the supernatant. 2. Remove and discard the fatty layer. 3. Transfer the cleared supernatant to a new tube.

3. Proceed to **Phase separation**, or store the homogenized sample. Homogenized samples can be stored at room temperature for several hours, or at –60 to –70°C for at least one month.

Phase separation

1. Incubate the homogenized sample (see **Homogenizing samples**) for 5 minutes at room temperature to permit complete dissociation of the nucleoprotein complex.
2. Add 0.2 mL of chloroform per 1 mL of TRIzol® Reagent used for homogenization. Cap the tube securely.
3. Shake tube vigorously by hand for 15 seconds.
4. Incubate for 2–3 minutes at room temperature.
5. Centrifuge the sample at 12,000 × *g* for 15 minutes at 4°C.

   Note: 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 upper aqueous phase is ~50% of the total volume.
6. Remove the aqueous phase of the sample by angling the tube at 45° and pipetting the solution out. Avoid drawing any of the interphase or organic layer into the pipette when removing the aqueous phase.
7. Place the aqueous phase into a **new** tube and proceed to the **RNA Isolation Procedure**.
8. Save the interphase and organic phenol-chloroform phase if isolation of DNA or protein is desired. See **DNA Isolation Procedure** and **Protein Isolation Procedure** for details. The organic phase can be stored at 4°C overnight.

RNA Isolation Procedure

Always use the appropriate precautions to avoid RNase contamination when preparing and handling RNA.

RNA precipitation

1. *(Optional)* When precipitating RNA from small sample quantities (<10^6 cells or <10 mg tissue), add 5–10 µg of RNase-free glycogen as a carrier to the aqueous phase.

   Note: Glycogen is co-precipitated with the RNA, but does not inhibit first-strand synthesis at concentrations ≤4 mg/mL, and does not inhibit PCR.
2. Add 0.5 mL of 100% isopropanol to the aqueous phase, per 1 mL of TRIzol® Reagent used for homogenization.
3. Incubate at room temperature for 10 minutes.
4. Centrifuge at 12,000 × *g* for 10 minutes at 4°C.

   Note: The RNA is often invisible prior to centrifugation, and forms a gel-like pellet on the side and bottom of the tube.
5. Proceed to **RNA wash**.

RNA wash

1. Remove the supernatant from the tube, leaving only the RNA pellet.
2. Wash the pellet, with 1 mL of 75% ethanol per 1 mL of TRIzol® Reagent used in the initial homogenization.

   Note: The RNA can be stored in 75% ethanol at least 1 year at –20°C, or at least 1 week at 4°C.
3. Vortex the sample briefly, then centrifuge the tube at 7500 × *g* for 5 minutes at 4°C. Discard the wash.
4. Vacuum or air dry the RNA pellet for 5–10 minutes. Do not dry the pellet by vacuum centrifuge.

   Note: Do not allow the RNA to dry completely, because the pellet can lose solubility. Partially dissolved RNA samples have an $A_{260/280}$ ratio <1.6.
5. Proceed to **RNA resuspension**.

RNA resuspension

1. Resuspend the RNA pellet in RNase-free water or 0.5% SDS solution (20–50 µL) by passing the solution up and down several times through a pipette tip.
   Note: Do not dissolve the RNA in 0.5% SDS if it is to be used in subsequent enzymatic reactions.
2. Incubate in a water bath or heat block set at 55–60°C for 10–15 minutes.
3. Proceed to downstream application, or store at –70°C.

DNA Isolation Procedure

DNA is isolated from the interphase and phenol-chloroform layer saved from the **Phase separation** step.

DNA precipitation

1. Remove any remaining aqueous phase overlying the interphase. This is critical for the quality of the isolated DNA.
2. Add 0.3 mL of 100% ethanol per of 1 mL TRIzol® Reagent used for the initial homogenization.
3. Cap the tube and invert the sample several times to mix.
4. Incubate samples for 2–3 minutes at room temperature.
5. Centrifuge the tube at 2000 × *g* for 5 minutes at 4°C to pellet the DNA.
6. Remove the phenol-ethanol supernatant and save it in a new tube if protein isolation is desired. The supernatant can be stored at –70°C for several months.
7. Proceed with the **DNA wash** step using the DNA pellet.

DNA wash

1. Wash the DNA pellet with 1 mL of sodium citrate/ ethanol solution (0.1 M sodium citrate in 10% ethanol, pH 8.5) per 1 mL of TRIzol® Reagent used for the initial homogenization.
2. Incubate for 30 minutes at room temperature. Mix occasionally by gentle inversion.
   Note: The DNA can be stored in sodium citrate/ethanol solution at least 2 hours.
3. Centrifuge at 2000 × *g* for 5 minutes at 4°C. Remove and discard supernatant.
4. Repeat wash (steps 1–3), once.
   Note: Repeat wash twice for large DNA pellets (>200 µg).
5. Add 1.5–2 mL 75% ethanol per 1 mL of TRIzol® Reagent used for the initial homogenization.
   Note: DNA samples may be stored in 75% ethanol at 4°C for several months.
6. Incubate for 10–20 minutes at room temperature. Mix the tube occasionally by gentle inversion.
7. Centrifuge at 2000 × *g* for 5 minutes at 4°C. Remove and discard supernatant.
8. Air or vacuum dry the DNA pellet for 5–10 minutes. Do not allow the pellet to dry out. Do not dry the pellet by vacuum centrifuge.
9. Proceed to the **DNA resuspension** step.

DNA resuspension

Resuspend the DNA in 8mM NaOH at a concentration of 0.2–0.3 µg/µL.

1. Add 0.3–0.6 mL of 8mM NaOH per 50–70 mg of tissue, or per 1×10^7 cells.
   Note: Resuspending the DNA is a mild base is highly recommended because isolated DNA does not resuspend well in water or Tris buffer.
2. Remove any insoluble material by centrifuging the sample at 12,000 × *g* for 10 minutes at 4°C.
3. Transfer the supernatant containing the DNA to a **new** tube. Adjust pH as needed with HEPES and proceed to downstream application of choice. The DNA can be stored overnight at 4°C, but for long-term storage adjust to pH 7–8 with HEPES, and add 1 mM EDTA. Store at 4°C or –20°C.

Determining Yield of RNA and DNA

Use absorbance of RNA and DNA at 260 nm and 280 nm to determine concentration.

Sample	Procedure
RNA	1. Dilute sample in RNase-free water, and measure absorbance at 260 nm and 280 nm. 2. Use the formula A_{260} × dilution × 40 = µg RNA/mL to determine concentration.
DNA	1. Dilute sample in water or buffer (pH >7.5), and measure absorbance at 260 nm and 280 nm. 2. Use the formula A_{260} × dilution × 50 = µg DNA/mL to determine concentration.

Expected yields

The table below presents typical yields of RNA ($A_{260/280}$ of >1.8) and DNA ($A_{260/280}$ of 1.6–1.8) from various starting materials.

Starting Material	Quantity	RNA	DNA
Epithelial Cells	1×10^6 cells	8–15 µg	—
New Tobacco Leaf	—	73 µg	—
Fibroblasts	1×10^6 cells	5–7 µg	5–7 µg
Cultured cells, mammal	1×10^6		5–7 µg
Skeletal muscles and brain Placenta	1 mg 1 mg	1–1.5 µg 1–4 µg	2–3 µg 2–3 µg
Liver Kidney	1 mg	6–10 µg 3–4 µg	3–4 µg 3–4 µg

Protein Isolation Procedure

Proteins are isolated from the phenol-ethanol supernatant layer left over after the **DNA precipitation** step. Isolate the protein using either **Protein precipitation** OR **Protein dialysis**.

Protein precipitation

1. Add 1.5 mL of isopropanol to the phenol-ethanol supernatant per of 1 mL TRIzol® Reagent used for the initial homogenization.
2. Incubate samples for 10 minutes at room temperature.
3. Centrifuge at 12,000 × *g* for 10 minutes at 4°C to pellet the protein. Remove and discard the supernatant.
4. Proceed to the **Protein wash** step with the remaining protein pellet.

Protein wash

1. Prepare a wash solution consisting of 0.3 M guanidine hydrochloride in 95% ethanol.
2. Wash the protein pellet with 2 mL of the wash solution per 1 mL of TRIzol® Reagent used for the initial homogenization.
3. Incubate for 20 minutes at room temperature.
   Note: Protein samples may be stored in 0.3 M guanidine hydrochloride-95% ethanol for at least one month at 4°C or for at least one year at –20°C.
4. Centrifuge at 7500 × *g* for 5 minutes at 4°C. Remove and discard the wash solution.
5. Repeat steps 2–4, two more times.
6. Add 2 mL of 100% ethanol to protein pellet after the third wash and vortex.
7. Incubate for 20 minutes at room temperature.
8. Centrifuge at 7500 × *g* for 5 minutes at 4°C. Remove and discard ethanol wash.
9. Air dry the protein pellet for 5–10 minutes. Do not allow the pellet to dry out.
10. Proceed to the **Protein resuspension** step.

Protein resuspension

1. Add 1% SDS to the protein pellet (200 µL) and pipet up and down until the protein is resuspend.
   Note: To completely dissolve the protein pellet, you may need to incubate the sample at 50°C in a water bath or heat block.
2. Centrifuge at 10,000 × *g* for 10 minutes at 4°C to sediment any insoluble material.
3. Transfer the supernatant containing the protein to a new tube and proceed to downstream application of choice, or store the sample at –20°C.

Protein resuspension, continued

Poor solubility of the pellet in SDS can occur, because the solubility of specific classes of proteins differs with different solvents. If the protein pellet is insoluble in SDS, the following alternative solvents (Hummon et. al., 2007) may be required to solubilize the pellet:

- 1% SDS and 62.5 mM sarkosyl at pH 8.0–8.8
- 9.5 M urea and 2% CHAPS, pH 9.1
- 250mM glycerol, 10mM TEA, and 4% CHAPS
- 2% diethylamine
- 10M Urea

Protein dialysis

1. Load the phenol-ethanol supernatant into the dialysis membrane.
   Note: The phenol-ethanol solution can dissolve some types of dialysis membranes (e.g., cellulose ester). Test dialysis tubing with the membrane to assess compatibility before starting.
2. Dialyze the sample against 3 changes of 0.1% SDS at 4°C. Make the first change of solution after 16 hours, the second change 4 hours later (at 20 hours), and the final change 2 hours later (at 22 hours).
   Note: 0.1% SDS is required to resolubilize the proteins from the pellet; a lower concentration of SDS is insufficient. If desired, the SDS can be diluted after solubilization.
3. Centrifuge the dialysate at 10,000 × *g* for 10 minutes at 4°C. Proteins are located in the clear supernatant.
4. Transfer supernatant to a new tube and proceed to downstream application, or store the sample at –20°C.
5. *(Optional)* Solubilize the pellet by adding 100 µL of 1% SDS and 100 µL of 8 M urea.

Determining Yield of Protein

Measure protein concentration by Bradford assay (SDS concentration must be <0.1%).

Troubleshooting

Problem	Cause	Solution
Low yield of RNA, Low yield of DNA, or Low yield of protein	• Incomplete homogenization or lysis of samples • Final RNA, DNA or protein pellet was incompletely redissolved	• Decrease the amount of starting material. Mince tissues into smaller pieces and make sure it is completely immersed in TRIzol® Reagent for optimal lysis. • Increase the solubilization rate by pipetting the sample repeatedly, and heat the sample to 50–60°C.
RNA is degraded, DNA is degraded, or Protein is degraded.	• Samples were not immediately processed or frozen after collection. • Isolated RNA, DNA, or protein preparations were stored at the incorrect temperature.	• Sample must be processed or frozen immediately after collection. • Store RNA samples at –60 to –70°C. Store DNA and protein samples at –20°C.
RNA contamination or DNA contamination	• Interphase/organic phase pipetted up with aqueous phase. • Incomplete removal of aqueous phase • DNA pellet insufficiently washed with 0.1 M sodium citrate in 10% ethanol	• Do not attempt to draw off the entire aqueous layer after phase separation. • Remove remnants of the aqueous phase prior to DNA precipitation. • Make sure pellet is washed with 0.1 M sodium citrate in 10% ethanol.
Low $A_{260/280}$ for RNA	• Sample was homogenized in an insufficient volume of TRIzol® Reagent • Incomplete removal of organic phase	• Add the appropriate amount of TRIzol® Reagent for your sample type (see **Homogenizing samples**). • Do not attempt to draw off the entire aqueous layer after phase separation.
Low $A_{260/280}$ for DNA	Phenol was not sufficiently removed from the DNA preparation	Wash the DNA pellet one additional time in 0.1 M sodium citrate in 10% ethanol.

References:

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

Chomczynski, P., and Sacchi, N. (1987) Single Step Method of RNA Isolation by Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction. Anal. Biochem. *162*, 156-159

Hummon, A. B., Lim S. R., Difilippantonio, M. J., Ried, T. (2007) Isolation and solubilization of proteins after TRIzol® extraction of RNA and DNA from patient material following prolonged storage. BioTechniques *42*, 467-472