

TrioMol Isolation Reagent



Technical Manual No. 0242

Version 06142007

I	Description.....	1
II	Key Features.....	1
III	Storage	1
IV	General Protocol Using Triomol Isolation Reagent.....	1
V	Troubleshooting	4
VI	Order Information.....	6

I. DESCRIPTION

TrioMol Isolation Reagent allows the isolation of total RNA, DNA, and protein from the same sample in a single-step liquid-phase separation. The procedure is an improvement of the single-step RNA isolation method developed by Chomczynski and Sacchi. This reagent performs well with both small and large quantities of tissue or cells. The cells may be of human, animal, plant, or bacterial origin.

II. KEY FEATURES

- Excellent performance of total RNA isolation from both tissue and cells
- Monophasic solution of phenol and guanidine thiocyanate for RNA, DNA, and protein isolation
- RNA, DNA, and protein isolation from the same sample in a single-step liquid-phase separation

III. STORAGE

Store at 2-8°C.

IV. GENERAL PROTOCOL USING TRIOMOL ISOLATION REAGENT

RNA isolation instructions:

Precautions for Preventing RNase Contamination:

- Wear clean disposable gloves at all times.
- Use sterile disposable plasticware and pipettes/tips reserved for RNA work only.

Reagents required but not supplied

- Chloroform
- Isopropyl alcohol
- 75% Ethanol (in DEPC-treated water)
- RNase-free water or 0.5% SDS (in DEPC-treated water)



1. Homogenization

a) Tissues

For each 50-100 mg of tissue to be processed, add 1 ml TrioMol Isolation Reagent to a polypropylene centrifuge tube at 15-25°C. The sample volume should not exceed 10% of the volume of TrioMol Isolation Reagent.

Note: 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. Clarify the homogenate by centrifuging it at 12,000 x *g* for 10 minutes at 2 to 8°C. Extracellular membranes, polysaccharides, and high molecular weight DNA pellet during the centrifugation, while excess fat collects as a layer on top of the supernatant. Remove the fatty layer (if any), then transfer the supernatant (which contains the RNA, most DNA, and protein) to a fresh polypropylene centrifuge tube.

b) Cells Grown in Monolayer

For 10 cm² area cells, add 1 ml TrioMol Isolation Reagent directly to the culture dish. Pass the cell lysate through a pipette several times. Transfer the cell lysate to a polypropylene centrifuge tube. Insufficient amount of TrioMol Isolation Reagent added may result in contamination of isolated RNA with DNA.

c) Cells Grown in suspension

Pellet cells by centrifugation. Lyse cells in TrioMol Isolation Reagent by repetitive pipetting. For 5-10×10⁶ animal, plant, or yeast cells; or 1×10⁷ bacterial cells, use 1 ml TrioMol Isolation Reagent.

2. Phase separation

Note: The sample obtained in procedure "1" can be stored at -60°C or below for at least 1 month before they are used in procedure "2".

Incubate each homogenized sample from procedure "1" for 5 minutes at 15 to 25°C to ensure the complete dissociation of nucleoprotein complexes. Add chloroform to each sample. Use 0.2 ml chloroform for each 1 ml TrioMol Isolation Reagent required in the initial homogenization. Cap tube securely, and shake it vigorously for 15 seconds. Incubate tube at 15 to 25°C for 2-15 minutes.

To separate the solution into three phases, centrifuge tube at 12,000 x *g* for 15 minutes at 2 to 8°C. After centrifugation, the mixture separates into a lower phenol-chloroform phase, an interphase and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase.

3. RNA precipitation

Transfer the aqueous phase to a fresh tube, and precipitate the RNA from the aqueous phase by mixing isopropyl alcohol. Use 0.5 ml isopropyl alcohol per 1 ml TrioMol Isolation Reagent used for the initial homogenization. Incubate samples at 15 to 30°C for 10 minutes and centrifuge at 12,000 x *g* for 10 minutes at 2-8°C.

4. RNA wash

Remove the supernatant. Wash the RNA pellet with 75% ethanol, using at least 1 ml of 75% ethanol per 1 ml TrioMol Isolation Reagent used in the initial homogenization. Mix the sample by vortexing and centrifuge at 7,500 x *g* for 5 minutes at 2-8°C.

5. Redissolving the RNA

Remove the excess ethanol from the RNA pellet by air-drying or placing the sample under vacuum for 5-10 minutes.



Note: Do not dry the RNA pellet by centrifugation under vacuum. Do not let the RNA pellet dry completely as a dry pellet will be much less soluble.

Resuspend the RNA pellet in DEPC-treated water or DEPC-treated 0.5% SDS. Dissolve the RNA pellet by passing the solution through a pipette tip several times, then incubating the solution for 10-15 minutes at 55°C to 60°C.

DNA isolation instructions:

Reagents required, but not supplied:

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

1. DNA precipitation

Remove the remaining aqueous phase overlying the interphase and precipitate the DNA from the interphase and organic phase with ethanol. Use 0.3 ml of 100% ethanol per 1 ml of TrioMol Isolation Reagent used in the initial homogenization. Mix samples by inversion. Keep the samples at 15 to 30°C for 2-3 minutes to allow the DNA precipitate to form. Centrifuge the sample at 2,000 × g for 5 minutes at 2-8°C.

Note: You must get rid of all the aqueous phase in order to obtain high quality DNA.

2. DNA wash

Remove the phenol-ethanol supernatant, 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 TrioMol Isolation Reagent used in the initial homogenization. At each wash, keep the DNA pellet in the washing solution for 30 minutes at 15 to 30°C (with periodic mixing) and centrifuge at 2,000 × 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 TrioMol Isolation Reagent), store for 10-20 minutes at 15 to 30°C (with periodic mixing) and centrifuge at 2,000 × g for 5 minutes at 2 to 8°C.

3. Redissolving DNA

Air dry the DNA 5 to 15 minutes in an open tube. (**Do not dry under centrifugation**; it will be more difficult to dissolve.) Dissolve DNA in 8 mM NaOH such that the concentration of DNA is 0.2-0.3 µg/µl. Typically add 300-600 µl of 8 mM NaOH to DNA isolated from 10⁷ cells or 50-70 mg of tissue.

Resuspending in weak base is highly recommended since isolated DNA does not resuspend well in water or in Tris buffers. The pH of the 8 mM NaOH is only ≈9 and should be easily adjusted with TE or HEPES once the DNA is in solution. At this stage, the DNA preparations (especially from tissues) may contain insoluble gel-like material (fragments of membranes, etc.). Remove the insoluble material by centrifugation at 12,000 × g for 10 minutes. Transfer the supernatant containing the DNA to a new tube.

DNA solubilized in 8 mM NaOH can be stored overnight at 4°C; for prolonged storage, samples should be adjusted with HEPES to pH 7-8 and supplemented with 1 mM EDTA. Once the pH is adjusted, DNA can be stored at 4°C or -20°C.



Protein isolation instructions:**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 supernatant with isopropyl alcohol. Add 1.5 ml of isopropanol per 1 ml of TrioMol Isolation Reagent used for the initial homogenization. Keep samples for 10 minutes at 15 to 30°C, and sediment the protein precipitate at $12,000 \times g$ for 10 minutes at 2 to 8°C.

2. Protein Wash

Remove the supernatant 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 TrioMol 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 $7,500 \times g$ 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 $7,500 \times g$ for 5 minutes at 2 to 8°C.

3. Redissolving the protein pellet

Remove the excess ethanol from the protein pellet by air-drying or placing the sample under vacuum 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 $10,000 \times g$ for 10 minutes at 2 to 8°C, and transfer the supernatant 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.

**V. TROUBLESHOOTING**

Table below is guideline for troubleshooting.

Procedure	Symptom	Problem Cause
RNA isolation	Low yield	• Incomplete homogenization or lysis of samples. • Final RNA pellet incompletely redissolved.
	A_{260}/A_{280} ratio <1.65	• RNA sample was diluted in water instead of TE prior to spectrophotometric analysis. Low ionic strength and low pH solutions increase absorbance at 280 nm. • Sample homogenized in too small volume TrioMol Isolation Reagent. • 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 or plant. • Samples used for isolation, or the isolated RNA preparations were stored at -4 to -20°C, instead of -60°C or below. • Cells were dispersed by trypsin digestion. • Aqueous solutions or tubes were not RNase-free.
	DNA contamination	• Sample homogenized in too small volume TrioMol Isolation Reagent. • Samples used for the isolation contained organic solvents (e.g., ethanol, DMSO), strong buffers, or alkaline solution.
DNA isolation	Low yield	• Incomplete homogenization or lysis of samples. • Final DNA pellet incompletely redissolved.
	A_{260}/A_{280} ratio <1.70	• DNA sample was diluted in water instead of TE prior to spectrophotometric analysis. • Phenol was not sufficiently removed from the DNA preparation.
	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 -4 to -20°C, instead of -60°C or below. • 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.
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.



VI. ORDER INFORMATION

TrioMol Isolation Reagent Cat. No.: M00105

For Research Use Only.

GenScript Corporation

120 Centennial Ave., Piscataway, NJ 08854

Tel: 732-885-9188

Fax: 732-210-0262, 732-885-5878

Email: info@genscript.com

Web: <http://www.Genscript.com>