TrioMol Isolation Reagent

Technical Manual No. 0242 **Version 06142007**

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I. DESCRIPTION

TrioMol Isolation Reagent is a monophasic solution of phenol and guanidine thiocyanate for RNA, DNA, and protein isolation. TrioMol Isolation Reagent isolates total RNA, DNA, and protein from sample mixtures in one single-step liquid-phase separation. The procedure is an improvement upon the single-step RNA isolation method developed by Chomczynski and Sacchi in which it 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 regarding 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°C to 8°C.

IV. GENERAL PROTOCOL USING TRIOMOL ISOLATION REAGENT

RNA isolation instructions:

Measures for the preventing of RNase contamination:

- Wear clean disposable gloves at all times.
- Use sterile disposable plasticware and pipettes or pipette 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°C to 25°C. The volume of the sample should not exceed 10% of the volume of the 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, or the tuberous parts of plants. Clarify the homogenate by centrifuging it at 12,000 X *g* for 10 minutes at 2°C to 8°C. Extracellular membranes, polysaccharides, and high-molecular-weight DNA pellet during the centrifugation, while

excess fat collects in a layer on top of the supernatant. Remove the fatty layer (if any), then transfer the supernatant (which contains the RNA, most of the DNA, and the protein) to a fresh polypropylene centrifuge tube.

b) Cells grown in monolayer
For every 10 cm^2 of 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 amounts of TrioMol Isolation Reagent may result in contamination of the isolated RNA with DNA.

c) Cells grown in suspension
Pellet cells by centrifugation. Lyse cells in TrioMol Isolation Reagent by repetitive pipetting. For every 5-10×10^6 animal, plant, or yeast cells or every 1×10^7 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 “I” for five minutes at 15°C to 25°C to ensure complete dissociation of nucleoprotein complexes. Add 0.2 ml of chloroform for each 1 ml TrioMol Isolation Reagent required in the initial homogenization. Cap the tube securely, and shake it vigorously for 15 seconds. Incubate tube at 15°C to 25°C for 2 to 15 minutes.

To separate the solution into three phases, centrifuge the tube at 12,000 X *g* for 15 minutes at 2°C to 8°C. After centrifugation, the mixture will separate 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°C to 30°C for 10 minutes and centrifuge at 12,000 X g for 10 minutes at 2°C to 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 five minutes at 2°C to 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 to 10 minutes.

Note: Do not dry the RNA pellet by centrifugation under vacuum. Do not let the RNA pellet dry completely, since 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 to 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°C to

30°C for 2 to 3 minutes to allow the DNA precipitate to form. Centrifuge the sample at 2,000 X *g* for 5 minutes at 2°C to 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. During each wash, keep the DNA pellet in the washing solution at 15°C to 30°C for 30 minutes (with periodic mixing) and centrifuge at 2,000 X *g* for five minutes at 2°C 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 to 20 minutes at 15°C to 30°C (with periodic mixing) and centrifuge at 2,000 X *g* for five minutes at 2°C to 8°C.
3. **Redissolving DNA**
   Air-dry the DNA 5 to 15 minutes in an open tube. (**Do not dry under centrifugation**. This will make the pellet more difficult to dissolve.) Dissolve the 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 10^7 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 those from tissues) may 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 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, the 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 at 15°C to 30°C for 10 mintutes, and sediment the protein precipitate at 12,000 X *g* for 10 minutes at 2°C to 8°C.
2. **Protein Wash**
   Remove the supernatant and wash the protein pellet three 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°C to 30°C and centrifuge at 7,500 X *g* for five minutes at 2°C 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°C to 30°C and centrifuge at 7,500 X *g* for five minutes at 2°C 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 to 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°C 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°C to -20°C for future use.

V. TROUBLESHOOTING

Use the table below to solve and avoid common problems.

Procedure	Symptom	Problem Cause
RNA isolation	Low yield	● Incomplete homogenization or lysis of samples. ● The final RNA pellet incompletely redissolved.
	A_{260}/A_{280} ratio <1.65	● The 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. ● The sample was homogenized in too small a volume of TrioMol Isolation Reagent. ● Following homogenization, the samples were not stored at room temperature for five 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. ● Either the samples used for isolation or the isolated RNA preparations were stored at -4°C to -20°C, instead of -60°C or below. ● The cells were dispersed by trypsin digestion. ● The aqueous solutions or tubes were not RNase-free.
	DNA contamination	● The sample was homogenized in too small a volume of TrioMol Isolation Reagent. ● The samples used for the isolation contain organic solvents (e.g., ethanol, DMSO), strong buffers, or alkaline solution.
DNA isolation	Low yield	● Incomplete homogenization or lysis of samples. ● The final DNA pellet is incompletely redissolved.
	A_{260}/A_{280} ratio <1.70	● The DNA sample was diluted in water instead of TE prior to spectrophotometric analysis. ● Phenol was not sufficiently removed from the DNA preparation.
	DNA degradation	● The tissues were not immediately processed or frozen after removal from the animal. ● Either the samples used for isolation or the isolated RNA preparations were stored at -4°C to -20°C, instead of -60°C or below. ● The samples were homogenized with a Polytron or some other high-speed homogenizer.
	RNA contamination	● Incomplete removal of aqueous phase. ● The DNA pellet is insufficiently washed with 0.1 M sodium citrate in 10% ethanol.
Protein Isolation	Low yield	● Incomplete homogenization or lysis of samples. ● The final DNA pellet is incompletely redissolved.
	Protein degradation	● The tissues were not immediately processed or frozen after removal from the animal.
	Band deformation in	● The protein pellet is insufficiently washed.

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VII. ORDERING INFORMATION

TrioMol Isolation Reagent Cat. No.: M00105

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