Cyrusbioscience

3-Zol (Trizol)

Cat. Number: 2001

Storage: Store at 4°C

Description

3-Zol 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. During sample homogenization or lysis, 3-Zol 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 phase. 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 ($5x10^6$), and large quantities of tissue (≥1g) and cells ($>10^7$), of human, animal, plant, or bacterial origin. The simplicity of the 3-Zol method allows simultaneous processing of large number of samples. The entire procedure can be completed in one hour. Total RNA isolated by 3-Zol 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 is recommended when the two primers lie within a single exon.

The isolated RNA has an A260/A280 ratio≥1.8 when diluted into TE buffer.

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.

- In the presence of 3-Zol, RNA is protected from RNase contamination. Downstream sample handling requires that non-disposable 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.
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Instructions for RNA isolation

Unless otherwise stated, the procedure is carried out at 15 to 30°C, and reagents are at 15 to 30°C.
Reagents required, but not supplied:

✧ Chloroform or Chloroform Replacement Separation Reagent (BCP)
✧ 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 water in 0.1% (v/v). Let it stir overnight and autoclave. The SDS solution must be prepared using DEPC-treated, autoclaved water.]

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.

1. HOMOGENIZATION

a. Tissues

Homogenize tissue samples in 1 ml of 3-Zol 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 3-Zol used for homogenization.

b. Cells grown in monolayer

Lyse cells directly in a culture dish by adding 1 ml of 3-Zol to a 3.5 cm diameter dish and passing the cell lysate several times through a pipette. The amount of 3-Zol added is based on the area of the culture dish (1 ml per 10 cm^2) and not on the number of cells present. An insufficient amount of 3-Zol may result in contamination of the isolated RNA with DNA.

c. Cells grown in suspension

Pellet cells by centrifugation. Lyse cells in 3-Zol by repetitive pipetting. Use 1 ml of the reagent per 5-10×10^6 of animal, plant or yeast cells, or per 1×10^7 bacterial cells. Washing cells before addition of

3-Zol 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.

OPTIONAL:

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 12,000x g for 10 minutes at 2 to 8°C. The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while the supernatant 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.

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 (or BCP) per 1 ml of 3-Zol. Cap sample tubes securely. Shake tubes vigorously by hand for 15 seconds and incubate them at 15 to 30°C for 2 to 3 minutes. Centrifuge the samples at no more than 12,000×g for 15 minutes at 2 to 8°C. Following centrifugation, the mixture separates into a lower green, phenol-chloroform phase, an 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 3-Zol 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 **cold (-20ºC) isopropyl alcohol**. Use 0.5 ml of isopropyl alcohol per 1 ml of 3-Zol used for the initial homogenization. Incubate samples at -20°C for 10 minutes and centrifuge at no more than 16,000×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 supernatant. Wash the RNA pellet once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of 3-Zol used for the initial homogenization. Mix the sample by vortex 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 5-10 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 A260/280 ratio < 1.6. Dissolve RNA in RNase-free water or 0.5% SDS solution by passing the solution

a few times through a pipette tip and incubating for 10 minutes at 55 to 60°C. (Avoid SDS when RNA will be used in subsequent enzymatic reactions.) RNA can also be re-dissolved in 100% formamide (deionized) and stored at -70°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 series of wash, the DNA is solubilized in 8mM NaOH. Full recovery of DNA from tissues and culture cells permits the use of 3-Zol Reagent for the determination of the DNA content in analyzed samples 2. Simultaneous extraction of genomic DNA allows for normalization of the results of Northern analysis per genomic DNA instead of the more variable 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
- ✧ 0.1M Sodium citrate in 10% ethanol
- ✧ 75% Ethanol
- ✧ 8mM NaOH

Unless otherwise stated, the procedure is carried out at 15~30^{o}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 0.3ml of 100% ethanol per 1ml of 3-Zol Reagent used for the initial homogenization, and mix samples by inversion. Next, store the samples at 15~30^{o}C for 2~3 minutes and sediment DNA by centrifugation at no more than 2,000x g for 5 minutes at 2~8^{o}C. Careful removal of the aqueous phase is critical for the quality of isolated 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 0.1M sodium citrate in 10% ethanol. Use 1ml of the solution per 1ml of 3-Zol Reagent used for the initial homogenization. At each wash, store the DNA pellet in the washing solution for 30 minutes at 15~30^{o}C (with periodic mixing) and centrifuge at 2,000x g for 5minutes at 2~8^{o}C. Following these two washes, resuspend the DNA pellet in 75% ethanol (1.5~2ml of 75% ethanol per 1ml 3-Zol Reagent), store for 10~20 minutes at 15~30^{o}C (with periodic mixing) and centrifuge at 2,000x g for 5 minutes at 2~8^{o}C. An additional wash in 0.1M sodium citrate-10% ethanol solution is required for large pellets, containing >200ug DNA large amounts of a non-DNA material.

3. REDISSOLVING THE DNA

Air dry the DNA 5~15 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 ug/ul. Typically add 300-600ul of 8mM 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 water or in Tris buffers. The pH of the 8mM NaOH is only ~9 and should be easily adjusted with TE buffer 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 >12,000g for 10minutes. Transfer the supernatant containing the DNA to a new tube. Stability of DNA in 8mM NaOH is several months at 4^{o}C; greater than one year at -20^{o}C; indefinitely at -70^{o}C.

4. QUANTITATION AND EXPECTED YIELDS OF DNA

Take an aliquot of the DNA preparation solubilized in 8mM 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 50ug of double-stranded DNA/ml. For calculation of cell number in analyzed samples, assume that the amount of DNA per 1×10^6 diploid cells of human, rat, and mouse origin equals: 7.1ug, 6.5ug, and 5.8ug, respectively3. The expected yield of DNA per mg of tissue is: 3~4ug from liver and kidney; and 2~3ug from skeletal muscles, brain and placenta, the expected yield of DNA per 1×10^6 cultured human, rat and mouse cells is 5~7ug.

Troubleshooting Guide

For RNA Isolation

Expected yields of RNA per mg of tissue or 1×10^6 cultured cells

Liver and spleen	6~10ug	Kidney	3~4ug
Skeletal muscles and brain	1~1.5ug	Placenta	1~4ug
Epithelial cells	8~5ug	Fibroblasts	5~7ug

- **Low yield**

Incomplete homogenization or lysis of samples.
Final RNA pellet incompletely re-dissolved.

- **A260/280 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 280nm. See Wilfinger, W. et. Al, Biotechniques 22:474-481. and Fox, D.K (1998) Focus 20:2 p.37). 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 ~ -20°C, instead of -60 ~ -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.

- **RNA contamination**

Sample homogenized in too a reagent volume. Sample 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 contamination compounds from the isolated RNA. Add to the aqueous phase 0.25ml of isopropanol followed by 0.25ml of a high salt precipitation solution (0.8M sodium citrate and 1.2M NaCl) per 1ml of 3-Zol 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 #2, RNA isolation protocol) is required to isolate pure from plant material containing a very high level of polysaccharides.

For DNA Isolation

Expected yields of DNA per mg of tissue or $1x10^6$ cultured cells

Liver and kidney	3~4ug	Fibroblasts	5~7ug	Skeletal muscles	2~3ug
Brain, and placenta	2~3ug	Cultured human, rat and mouse cells		5~7ug	

- **Low yield**

Incomplete homogenization or lysis of samples.
Final DNA pellet incompletely re-dissolved.

- **A260/280 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. Wash the DNA pellet and additional time with 0.1M sodium citrate in 10% ethanol.

- **DNA degradation**

Tissues were not immediately processed or frozen after removal from the animal. Sample used for isolation, or the isolated RNA preparations were stored at -5 ~ -20°C, instead of -60 ~ -70°C. Sample were homogenized with a Polytron or other high-speed homogenizer.

- **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 ug 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.