RNA-*Solv*® Reagent Concentrate

Omega Bio-tek

RNA Isolation Solvent

Before use: *This reagent supplied as concentrate, please add* ***water satured phenol*** *before use!*

R6830-00IN	*R6830-01IN*	*R6830-02IN*
Add 4.5 mL of water saturated phenol	*Add 45 mL of water saturated phenol*	*Add 90 mL of water saturated phenol*

Product No. R6830-00IN (5.5 ml)
Product No.R6830-01IN (55 ml)
Product No.R6830-02IN (110 ml)
Storage Conditions: RNA-*Solv*® is stable for at least 24 months when stored at 2°C-8°C and yields reproducible results.

Prepare the water saturated phenol: Dissolve the bottle contains solid phenol in a water bath at 65-75°C. Add equal volume of water and shake vigorously for 1 minutes. Leave the bottle at 4°C overnight or until two phase clearly separated. Remove the water phase and use the phenol to make working solution for RNA-*Solv*® Reagent.

Introduction
RNA-*Solv*® Reagent is a one reagent system for the isolation of total RNA from cells and tissues. The reagent, a single-phase solution consisting of phenol and guanidine isothiocyanate, is modification of the single-step RNA isolation method developed by Chomczynski and Sacchi (1).The sample is homogenized and lysed in RNA-*Solv*® Reagent which maintains the integrity of the RNA, while disrupting and denaturing endogenous RNases and other cellular components. Extraction of the lysate with chloroform further denatures proteins and separates the mixture into an organic and an aqueous phase. RNA remains exclusively in the aqueous phase, and is subsequently recovered by isopropanol.

This method is suitable for small quantities of tissue (<100 mg) and cells (<5 X10^6), and large quantities of tissue (up to1 g) and cells (<10^8), of human, animal, plant, or bacterial origin. The simplicity of the RNA-*Solv*® Reagent method allows simultaneous processing of a large number of samples. The entire procedure can be completed in one hour. Total RNA prepared in this manner 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 amplification by thermal cycling, treatment of the isolated RNA with RNase-free DNase I is recommended when the two amplimers lie within a single exon.

Supplied By User:

- Chloroform (no isoamyl alcohol added)
- Isopropyl alcohol
- 80% Ethanol (in DEPC-treated water)
- RNase-free water
- Tabletop centrifuge capable of 12,000 x g at room temperature

General Notes Regarding RNase Contamination

Whenever working with RNA :

- Always wear disposable gloves and change gloves frequently.
- Use sterile, disposable plasticware and automatic pipettes reserved for RNA work to prevent cross-contamination with RNases.
- In the presence of RNA-*Solv*® Reagent, RNA is protected from RNase contamination. Downstream sample handling requires that *nondisposable* glassware or plasticware be RNase-free.
- Use only DEPC-treated buffers. Add DEPC to a final concentration of 0.1%, incubate at 37°C for 2 hours, and autoclave at 121°C. Do not add DEPC to Tris buffers. Such buffers must be prepared by using DECP-water.

Precaution
Use only disposable polypropylene tubes for small samples and glass Corex tubes for larger samples. All tubes must be able to withstand 12,000 x g . Polystyrene tubes may crack with chloroform

Before Starting
A. Small Samples :To isolate RNA from very small samples (<10^6 cells or <10 mg tissue) perform homogenization (or lysis) of samples in 0.8 mL of RNA-*Solv*®, and add 1 mg RNase-free glycogen or yeast tRNA as carrier. This will improve yields obtained with precipitation.

B. Difficult Animal Samples: Specimens containing large amounts of proteins, fat, polysaccharides or extracellular material such as muscles, fat tissue, and sperm, will require the following modification. After lysis/homogenization in RNA-*Solv*® Reagent, centrifuge at 12,000 x g for 10 minutes at room temperature to remove insoluble debris. Often a precipitate forms at the bottom of the tube, but with fatty tissue, a lipid layer will also form above the aqueous phase. The supernatant will contain the RNA and must be carefully transferred to a fresh 1.5 ml microfuge tube before proceeding.

C. Interruption the procedure: Following lysis in RNA-*Solv*® Reagent and before addition of chloroform, samples can be stored at -70°C for up to 3 months. In addition, once the RNA is precipitated in isopropanol, the pellet may be stored at -20°C or -70°C for up to 1 year.

RNA-*Solv*® Protocol for Total RNA Isolation

CAUTION: When working with RNA-*Solv*® Reagent use gloves and eye protection (safety goggles) and avoid contact with skin or clothing. Work in a chemical fume hood to avoid inhaling vapor. Unless otherwise noted, all steps are to be carried out at room temperature (20°C-25°C).

1. Homogenization and lysis of samples: follow either method below

a) Tissue Samples
Homogenize tissue samples in 1 mL of RNA-*Solv*® Reagent per 50-100 mg of tissue using an appropriate mechanical homogenizer. Alternatively one can pulverize tissue in liquid nitrogen with mortar and pestle and transfer the powder to a clean 1.5 ml microcentrifuge tube. If ceramic mortar and pestle are not available, homogenize the sample in the microfuge tube using a disposable microtube pestle (Eppendorf, Cat No. 0030 120.973; VWR, Cat No. KT 749520-0000). The sample volume should not exceed 10% of the volume of RNA-*Solv*® Reagent used.

b) Cells Grown in Suspension
Pellet cells by centrifugation. Lyse cells in RNA-*Solv*® Reagent by repetitive pipetting. Use 1 mL of the reagent per 5-10 x 10^6 of

animal, plant or yeast cells, or per 1 x 10^8 bacterial cells. Washing cells before addition of RNA-*Solv*® Reagent should be avoided as this increases the possibility of mRNA degradation and RNase contamination. For plant, fungal, and yeast cells mechanical or enzymatic homogenization may be required. Also, for plant, fungal, and yeast cells, we recommend the use of the E.Z.N.A.® Plant (R6627),Fungal (R6640), and Yeast (R6670) RNA Kits from Omega Bio-tek.

c) Cells Grown in Monolayer
Lyse cells directly in a culture dish by adding 1 mL of RNA-*Solv*® Reagent to a 3.5 cm diameter dish, and passing the cell lysate several times through a blue pipette tip. The amount of RNA-*Solv*® Reagent added is based on the area of the culture dish (~1 mL per 10 cm^2). An insufficient amount of RNA-*Solv*® Reagent may result in contamination of the isolated RNA with DNA. Always use more RNA-*Solv*® Reagent if in the lysate is too viscous to aspirate with a pipette.

2. Add 0.2 mL of chloroform per 1 mL of RNA-*Solv*® Reagent. Cap sample tubes securely and vortex vigorously for 15 seconds. Incubate on ice for 10 minutes. *This step is critical - do not change it.*

3. Centrifuge the samples at no more than 12,000 x g for 15 minutes at room temperature. The mixture separates into a lower phenol-chloroform phase, an interphase, and an upper aqueous phase. RNA remains entirely in the aqueous phase.

4. **Precipitation of RNA.** Transfer no more than 80% of the aqueous phase to a fresh tube, and discard the lower organic phase. Precipitate the RNA from the aqueous phase by adding 500 µl of isopropyl alcohol per 1 mL of RNA-*Solv*® Reagent used for the initial homogenization. Incubate samples at room temperature 10 minutes and centrifuge at no more than 12,000 x g for 10 minutes also at room temperature.

Carbohydrate-rich samples: Plant samples of high polysaccharide content or animal tissues rich in glycosaminoglycans (proteoglycans) require the following modified precipitation method for obtaining pure RNA. Prepare Buffer A (1.2 M sodium chloride, 800 mM sodium citrate). Following step 3, add to the aqueous phase 0.3 ml isopropanol followed by 0.3 ml Buffer A per 1 ml RNA*Solv* ® Reagent used in step 1. Vortex to mix and centrifuge at no more than 12,000 x g for 10 minutes at room temperature. This high salt precipitation will reduce co-purification of complex carbohydrates.

5. **Wash RNA pellet.** Discard the supernatant and wash the RNA pellet once with 1 ml 80% ethanol. Mix the sample by vortexing and centrifuge at no more than 7,500 x g for 5 minutes at room temperature.

4. **Reconstitute RNA.** Carefully aspirate and discard the ethanol and briefly **AIR DRY** the RNA pellet for 2-5 minutes at room temperature.10 minutes). Do not use centrifugal devices equipped with a vacuum source as over-drying will lead to difficulty in re-dissolving RNA in water. Dissolve RNA in RNase-free water - a 5 minute incubation at 60oC may be required. RNA can also be reconstituted in 100% formamide (deionized) and stored at -70°C.

RNA is now suitable for RNase protection, northern analysis and reverse transcriptase reactions. For isolation of poly(A)+ RNA an additional ethanol precipitation is required. Add 1/8 X volume of RNase-free 3M NaOAc, pH 6.0 followed by 2.5 X volume absolute ethanol. Vortex to mix and incubate at room temperature for 5 minutes. Centrifuge at 12,000 x g for 10 min at room temperature and discard the supernatant. Wash the pellet as before and reconstitute in DECP-treated water.

Determination of Yield and Quality

UV spectrophotometric analysis of the purified RNA is required for obtaining yield. To do so, dilute the RNA in an appropriate volume of TE buffer, pH 8.0 (not water; RNA yields low Abs ratio values if dissolved in acidic buffers) and measure absorbance at 260 nm and at 280 nm.

RNA Conc = 40 µg/ml X Dilution factor X Abs 260 nm

Typical Abs 260 nm/ 280 nm ratios of 1.7-1.9 are obtained with the protocol. Yields vary depending of type and amount of starting material, and on condition of storage prior to processing. For assessing the quality of RNA, we recommend you perform denaturing agarose gel electrophoresis to confirm the integrity of purified material. Invariably, the full spectrum of RNAs, including 4S and 5S species are purified with RNA-*Solv*® Reagent.

Expected Yields per 1 mg tissue or 10^6 cells:

Liver and spleen, 5-10 µg
Kidney, 2-5 µg
Brain, 1-2 µg
Endothelial cells, 7-12 µg
Fibroblasts, 6-8 µg

Troubleshooting

- **Low RNA Yields:** Incomplete lysis of samples in RNA*Solv* Reagent. RNA pellet not completely dissolved in DEPC-water. pH of diluent used for spectrophotometric analysis is too low.
- **Degraded RNA:** Tissues were not immediately processed or frozen. Inadequate storage of starting material prio to isolation. Inadequate storage of RNA (-5 to -20°C, instead of -60 to -70°C) Trypsin/EDTA was used in dislodging monolayer cells. Buffers or plasticware were not RNase-free. Formaldehyde used for denaturing agarose-gel electrophoresis had a pH below 3.0.
- **Low Abs260/Abs280 ratios:** Sample was diluted in water rather than TE. Acidic pH lowers absorbance ratios. Use TE buffer as diluent for readings. Insufficient RNASolv Reagent was used for lysis of sample. Ice incubation in step 2 was not performed. The aqueous phase was contaminated with the phenolic phase.
- DNA contamination of RNA: Too little RNASolv Reagent used for sample processing causing inadequate separation of DNA/nucleoprotein complexes from aqueous RNA. The aqueous phase was contaminated with the phenol phase.

References:
1. Chomczynski, P., and Sacchi, N. Anal. Biochem. 162, 156 (1987).
2. Chomczynski, P. Biotechniques 15, 532 (1993).

For laboratory research use only.
CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.