RNA-Solv® Reagent

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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 a wide range of starting material; up to 1 g of tissue or 1 x 10^8 cells 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 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.

New in this Edition: This manual has been edited for content and redesigned to enhance user readability.

Kit Contents

Product	R6830-01	R6830-02	R6830-03
Preparations	100 preps	200 preps	500 preps
RNA-Solv® Reagent	100 mL	200 mL	2 x 250 mL
User Manual	✓	✓	✓

Storage and Stability

RNA-Solv® Reagent is stable for at least 24 months when stored at 2-8°C.

Important Notes

WARNING: This reagent is toxic if swallowed and can causes burns when in contact with skin. After contact with skin, wash immediately with copious amounts of mild detergent and water. If you feel sick, seek medical advice at once and Quote UN2821.

Please take a few minutes to read this booklet in its entirety to become familiar with the procedures. Prepare all materials required before starting to minimize RNA degradation.

- Whenever working with RNA, always wear gloves to minimize RNase contamination.
- Use sterile, disposable plastic ware 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 non-disposable glassware or plastic ware be RNase-free.
- Work quickly, but carefully.
- Use only DECP-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.
- All centrifugation steps must be carried out at room temperature.
- Prepare all materials required before starting the procedure to minimize RNA degradation.

Expected Yields

Expected RNA yield per 1 mg tissue or 1 x 10^6 cells can be found in the table below.

Tissue Source	Expected RNA Yield
Liver or Spleen	5-10 µg
Kidney	2-5 µg
Brain	1-2 µg
Endothelial Cells	7-12 µg
Fibroblasts	6-8 µg

Quantification and Storage of RNA

To determine the concentration and purity of RNA, measure absorbance at 260 nm and 280 nm with a spectrophotometer. One OD unit measured at 260 nm corresponds to 40 µg/mL RNA. DEPC-treated water is slightly acidic and can dramatically lower absorbance values. We suggest that you dilute the sample in a buffered solution (TE) for spectrophotometric analysis. The A_{260}/A_{280} ratio of pure nucleic acids is 2.0, while an A_{260}/A_{280} ratio of 0.6 denotes pure protein. A ratio of 1.8-2.0 corresponds to 90%-100% pure nucleic acid. Phenol has a maximum absorbance at 270 nm and can interfere with spectrophotometric analysis of DNA or RNA. Store RNA samples at -70°C in water. Under these conditions, RNA is stable for more than a year.

RNA Concentration = 40 µg/mL x Dilution factor x Abs 260 nm

Integrity of RNA

It is highly recommended that RNA quality be determined prior to beginning all downstream applications. The quality of RNA can be best assessed by denaturing agarose gel electrophoresis with ethidium bromide staining. The ribosomal RNA bands should appear as sharp, clear bands on the gel. The 28S band should appear to be double that of the 18S RNA band (23S and 16S if using bacteria). If the ribosomal RNA bands in any given lane are not sharp and appear to be smeared towards the smaller sized RNA, it is very likely that the RNA undergone degradation during the isolation, handling, or storage procedure. RNA-Solv® Reagent isolates the full spectrum of RNA, including 4S and 5S species.

RNA-Solv® Reagent Protocol - 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.

Materials and Reagents to be Supplied by User:

- Chloroform (no isoamyl alcohol added)
- Isopropyl alcohol
- 80% ethanol: diluted in nuclease-free water
- Nuclease-free water
- Tabletop centrifuge capable of 12,000 x *g*
- Nuclease-free 1.5 mL microcentrifuge tubes (Cat# SSI-1210-00)
- Mechanical homogenizer, mortar and pestle, or 1.5 mL homogenization pestle (Cat#SSI-1015-39)
- Vortexer
- Optional: DEPC Water (Cat# PR032)
- Optional: 100% ethanol
- Optional: RNase-free 3M NaOAc

1. Homogenization and lysis of samples. Choose one method below based on the starting material.

    - **Tissue Samples**

        1. Homogenize tissue samples in 1 mL RNA-Solv® Reagent per 50-100 mg of tissue using an appropriate mechanical homogenizer.

            Note: Alternatively one can pulverize tissue in liquid nitrogen with a mortar and pestle. Transfer the powder to a clean 1.5 ml microcentrifuge tube. If a mortar and pestle are not available, homogenize the sample in the microcentrifuge tube using a disposable 1.5 mL pestle. The sample volume should not exceed 10% of the volume of RNA-Solv® Reagent used.

    - **Cells Grown in Suspension**

        1. Pellet cells by centrifugation. Do not wash cells.

            Note: Washing cells before addition of RNA-Solv® Reagent should be avoided as this increases the possibility of mRNA degradation and RNase contamination.

2. Add 1 mL RNA-Solv® Reagent to 5-10 x 10^6 of animal, plant, or yeast cells, or to 1 x 10^8 bacterial cells.

   Tip: For plant, fungal, and yeast cells, mechanical or enzymatic homogenization may be required. Alternatively, we recommend the use of the E.Z.N.A.® Plant (Cat# R6827), Fungal (Cat# R6840), and Yeast (Cat# R6870) RNA Kits from Omega Bio-tek.

- **Cells Grown in Monolayer**

  1. Add 1 mL RNA-Solv® Reagent directly to a 3.5 cm diameter culture dish and pipette to lyse the cells.

     Note: The amount of RNA-Solv® Reagent required 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 DNA contamination of the isolated RNA. Always use more RNA-Solv® Reagent if the lysate is too viscous to aspirate with a pipette.

2. Add 0.2 mL chloroform per 1 mL RNA-Solv® Reagent. Cap sample tubes securely and vortex vigorously for 15 seconds.

3. Incubate on ice for 10 minutes. **This step is critical. Do not change it.**

Important Optional Protocol: Plant samples of high polysaccharide content or animal tissues rich in glycosaminoglycans (proteoglycans) require the following modified precipitation method for obtaining pure RNA. This high salt precipitation will reduce co-purification of complex carbohydrates. **If this optional protocol is not needed, continue to the centrifugation step in Step 4 of the main protocol below.**

1. Prepare Buffer A (1.2 M sodium chloride, 800 mM sodium citrate).

2. Add 0.3 mL isopropyl alcohol per 1 mL RNASolv® Reagent used in Step 1 to the aqueous phase.

3. Add 0.3 mL Buffer A per 1 mL RNASolv® Reagent used in Step 1.

4. Vortex to mix thoroughly.

5. Centrifuge at ≤12,000 x *g* for 10 minutes.

   Note: The mixture separates into a lower phenol-chloroform phase, an interphase, and an upper aqueous phase. RNA separates into the aqueous phase.

6. Continue to **Step 5** below.

4. Centrifuge at ≤12,000 x *g* for 15 minutes.

   Note: The mixture separates into a lower phenol-chloroform phase, an interphase, and an upper aqueous phase. RNA separates into the aqueous phase.

5. Transfer no more than 80% of the aqueous phase to a fresh microcentrifuge tube.

6. Add 500 µL isopropyl alcohol per 1 mL RNA-Solv® Reagent used in Step 1.

7. Incubate samples for 10 minutes.

8. Centrifuge at ≤12,000 x *g* for 10 minutes.

9. Discard the supernatant.

10. Add 1 mL 80% ethanol. Vortex to mix thoroughly.

11. Centrifuge at ≤7,500 x *g* for 5 minutes.

12. Carefully aspirate and discard the ethanol.

13. Air dry the RNA pellet for 2-5 minutes. Do not use centrifugal devices equipped with a vacuum source as over-drying will lead to difficulty in re-dissolving RNA in water.

14. Add 50-75 µL nuclease-free water or DEPC Water. Store RNA at -70°C.

    Note: A 5 minute incubation at 60°C may be required. RNA can also be reconstituted in 100% formamide (deionized).

Optional: For isolation of poly(A)+ RNA, an additional ethanol precipitation is required.

1. Add 1/8 x volume RNase-free 3M NaOAc, pH 6.0.
2. Add 2.5 x volumes 100% ethanol.
3. Vortex to mix thoroughly.
4. Incubate for 5 minutes.
5. Centrifuge at 12,000 x *g* for 10 minutes.
6. Discard the supernatant.
7. Repeat Steps 10-14 beginning with the addition of 80% ethanol on the main protocol above.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause
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 prior to isolation or RNA.
	Trypsin/EDTA was used in dislodging monolayer cells.
	Buffers or plastic ware 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 RNA-Solv® Reagent was used for lysis of sample.
	Ice incubation in Step 3 was not performed.
	The aqueous phase was contaminated with the phenolic phase.
DNA Contamination of RNA	Inadequate RNA-Solv® Reagent was used causing poor 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).

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

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
DEPC Water, 100 mL	PR032
Homogenization Pestles, 1.5 mL, 10/bag, 20 bags/cs	SSI-1015-39

Notes: