RNAzol™ B ISOLATION OF RNA

U.S. PATENT | **For in vitro research use** | **TEL-TEST BULLETIN NO. 2**

1. INTRODUCTION

With the extensive research in the field of molecular biology the isolation of pure, undegraded RNA from a large number of biological samples has become a critical issue. RNAzol™ is an easy answer to this complicated problem. Various methods have been used for isolation of undegraded RNA (3,4). Recent progress in this field has made it possible to convert previous lengthy and laborious methods of RNA isolation into a single-step procedure (1). Cinna/TEL-TEST has modified and refined this procedure creating the most reliable and advanced method of RNA isolation. The RNAzol™ and RNAzol™ B methods, which have been formulated as a result of extensive laboratory investigation, are both based on the unique property of RNAzol™ which promotes formation of complexes of RNA with guanidinium and water molecules, and abolishes hydrofilic interactions of DNA and proteins. In effect, DNA and proteins are efficiently removed from the aqueous phase, while RNA remains in this phase during the sample extraction with RNAzol™.

The RNAzol™ method can be completed within 3 hours, and the RNAzol™ B method within 1.5 hour. Both methods provide the same high yield and purity of RNA preparations. The simplicity of the RNAzol™ methods and excellent recovery of RNA from small quantities of tissue or cells makes our products especially suitable for gene expression studies for which only a limited quantity of biological material is available. The RNAzol™ is undegraded, free of DNA and proteins and contains the whole spectrum of RNA molecules, including small (4-5 S) RNAs. The preparation is ready for dot blot hybridization, gel electrophoresis to detect specific mRNA by Northern blotting, poly A+ selection by the oligo dT-cellulose method, or may be used for molecular cloning, in vitro translation, PCR*, RNase protection and other enzymatic assays. The RNAzol™ methods may also be used for rapid and efficient removal of DNA from RNA probes used in hybridization assays.

2. REAGENTS SUPPLIED

RNAzol™ B:	1 bottle (100 mL or 200 mL) containing a solution of RNAzol™ B
Preparation:	Ready to use.
Storage:	Refrigerate at 2-8° C. Do not freeze.
Stability:	Refer to expiration date on the bottle (Stable up to nine months).

3. REAGENTS REQUIRED, BUT NOT SUPPLIED

Chloroform (ACS grade)

Isopropanol (ACS grade)

75% Ethanol (ACS grade)

4. METHOD

RNA isolation by the RNAzol™ B method includes the following steps:

1. Homogenization	RNAzol™ B (2 mL/100 mg tissue or 10x10^6 cells)
2. RNA Extraction	1 vol. homogenate +0.1 vol. Chloroform
3. RNA Precipitation	1 vol. isopropanol
4. RNA Wash	75% ethanol

Unless stated otherwise the procedure is carried out at room temperature.

4.1 HOMOGENIZATION

(a) Homogenize tissue samples with RNAzol™ B (2 mL per 100 mg tissue) with a few strokes in a glass-Teflon homogenizer.

(b) To isolate RNA from cells grown in suspension, sediment cells and lyse them by the addition of 0.2 mL of RNAzol™ B per 10^6 cells. Cells grown in monolayer are lysed directly in the culture dish by the addition of RNAzol™ B [1 mL per 3.5 cm petri dish or 10 cm^2 (ex. T-30 flask = 30 cm^2 = 3mL)]. Solubilize RNA by passing the lysate a few times through the pipette.

4.2 RNA EXTRACTION

Add 0.2 mL chloroform per 2 mL of homogenate, cover the samples tightly, shake vigorously for 15 seconds (do not vortex) and let them stay on ice (or at 4° C) for 5 minutes. Centrifuge the suspension at 12,000g (4° C) for 15 minutes. After addition of chloroform and centrifugation, the homogenate forms two phases: the lower blue phenol-chloroform phase and the colorless upper aqueous phase whereas DNA and proteins are in the interphase and organic phase. A volume of the aqueous phase is about 50% of the initial volume of RNAzol™ B plus a volume of tissue used for homogenization.

4.3 RNA PRECIPITATION

Transfer the aqueous phase to the fresh tube, add an equal volume of isopropanol and store the samples for 15 minutes at 4° C. Centrifuge samples for 15 minutes at 12,000 g (4° C). RNA precipitate (often invisible before centrifugation) forms a white-yellow pellet at the bottom of the tube.

4.4 RNA WASH

Remove the supernatant and wash the RNA pellet once with 75% ethanol by vortexing and subsequent centrifugation for 8 minutes at 7,500 g (4° C or -20° C). Use at least 0.8 mL of ethanol per 50-100 µg RNA.

At the end of the procedure, dry the pellet briefly under vacuum for 10-15 minutes. It is important not to let the RNA pellet dry completely, as it will greatly decrease its solubility. Dissolve the RNA pellet in 0.5% SDS or in 1mM EDTA, pH 7 solution by vortexing or by passing the RNA solution a few times through a pipette tip. An incubation for 10-15 minutes at 60°C may be required to dissolve preparations of RNA. Diethylpyrocarbonate (DEPC) - treated RNase free solutions (3) should be used for RNA solubilization.

The final preparation is free of DNA and proteins and has a 260/280 ratio higher than 1.9.

5. NOTES AND COMMENTS

5.1 Isolation of RNA from a small amount of tissue (1-10 mg): Homogenize samples in 0.8 mL of RNAzol™ B, transfer the homogenates to Eppendorf tubes, add 80 µL of chloroform and store samples for 5 minutes at 4° C. Centrifuge samples in an Eppendorf centrifuge for 15 minutes, collect the aqueous phase and precipitate RNA with 0.4 mL of isopropanol for 45 minutes or overnight at 4° C. Centrifuge RNA precipitates for 15 minutes and wash once with 0.8 mL of 75% ethanol.

5.2 Isolation of RNA from blood, serum and other liquid matrices: Homogenize samples in 10 - 15mL of RNAzol B per 1mL of sample.

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5.3 Isolation of RNA from bacterial cells. homogenize samples in 10 - 15mL of RNAzol B per 1mL pellet of bacterial cells.

5.4 Following isopropanol addition, store samples overnight at 4° C in case the procedure has to be interrupted at this step.

5.5 An additional precipitation is necessary to use RNA isolated by the RNAzol™ B method in enzymatic assays. Following solubilization, precipitate RNA in the presence of 0.2 M NaCl with one volume of isopropanol or with two volumes of ethanol for 1 hour at -20° C.

5.6 Hands and dust may be the major source of RNase contamination. Use gloves and keep tubes closed. The use of sterile, disposable polypropylene tubes is recommended throughout the procedure.

5.7 Some commercial SDS preparations have acid pH. Adjust SDS solution to pH 6.5 - 7.5 if necessary.

6. SPECIAL HANDLING PRECAUTIONS

RNAzol™ B contains an irritant (guanidinium thiocyanate) and poison (phenol).

Handle RNAzol™ B work with gloves. Do not get in eyes, skin, or clothing. Avoid breathing vapor.

In case of contact: Immediately flush eyes or skin with a large amount of water for at least 15 minutes and seek immediate medical attention.

Read the warning note on the bottle.

TROUBLESHOOTING GUIDE

PROBLEM	CAUSE	SOLUTION
Lower than expected 260/280	Over dried pellet.	Do not use speed vac. Dry pellet briefly at room temperature. During wash step centrifuge at 7,500g max. Incubate RNA pellet 10 - 15 minutes at 60° C.
	Contamination of aqueous layer with interphase/organic phase	Take less of aqueous phase. Use small bore pipet tips. Exercise care while removing aqueous layer.
	Sample contains glycogen, polysaccharides or other contaminants	Wash pellet in 4M LiCL[2] prior to ethanol wash.
	Vortexing	Always hand shake samples during extraction.
	Trouble with spectrophotometer or spectrophotometric method	Check spectrophotometric method.
	Dilution of RNAzol B with sample	Sample volume should not exceed 10% of the volume of RNAzol B used for homogenization.
DEGRADED RNA	Endogenous RNase Activity	Use fresh tissue or cells.
	Exogenous RNase contamination	See section 5.6 in notes and comments section.
	Homogenization Step extended beyond 20 minutes.	Extract samples within 20 minutes. For multiple samples freeze homogenates at -70° C for later simultaneous processing.
DNA CONTAMINATION	Contamination of aqueous phase with interphase/organic phase	Take less of aqueous phase. Use small bore pipet tips. Exercise care while removing aqueous phase.
	Dilution of RNAzol B with sample	Sample volume should not exceed 10% of the volume of RNAzol B used for homogenization.

REFERENCES

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4. R. A. Cox in *Methods in Enzymology* (L Grossman and K. Moldave, Eds.) vol.12, part B, pp. 120-129, Academic Press, Orlando, FL (1968).

* Polymerase Chain Reaction (PCR) technology is covered by U.S. Patents issued to Cetus Corporation.

RNAzol™ B

Catalog No. CS-104 100 mL $85.00 | Catalog No. CS-105 200mL $150.00

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