

Ultraspec™ RNA

ISOLATION SYSTEM

For Laboratory Use

A new and improved method for the isolation of total RNA from Tissues/Cells/Bacteria/Plant and Yeast.

II. INTENDED USE

For isolation and purification of total RNA from tissues, cells, bacteria, plant, and yeast.

II. INTRODUCTION

Isolation of high quality RNA is one of the most challenging techniques in modern molecular biology. Recent progress in RNA isolation technology has made it possible to replace lengthy and laborious methods of total RNA isolation by a single-step method. Biotecx continues to modify and refine these procedures offering the most reliable and advanced RNA isolation products. Ultraspec™ RNA isolation method for total RNA is a new and substantially improved version of the single-step methods currently available from Biotecx. This method is based on the principle of a formulation of a 14 M solution of guanidine salts and urea (Chaosolv) which act as denaturing agents. The Chaosolv in conjunction with phenol and other detergents is found to be a very effective reagent for the isolation of total RNA from tissues and cells of human, animal, and bacterial origin. Ultraspec™ RNA is highly reliable and produces very consistent results.

The entire procedure for RNA isolation using the Ultraspec™ RNA method can be completed in less than 1 hour. A biological sample is homogenized with Ultraspec™ RNA using a glass-teflon or polytron homogenizer. The homogenate separates into two phases after extraction with chloroform. The total RNA remains exclusively in the aqueous phase while DNA and proteins are extracted into an organic phase and interphase. The total RNA is precipitated from the aqueous phase by addition of isopropanol, washed with ethanol and solubilized in DEPC treated water.

III. APPLICATION

The total RNA isolated by this method is undegraded and free of protein and DNA contamination. It can be used for Northern analysis, dot blot hybridization, poly A+ selection, in vitro translation, RNase protection assay, molecular cloning, and for reverse transcriptase/polymerase chain reaction (PCR*) without additional treatment with DNase. The simplicity of the isolation using the Ultraspec™ RNA makes it possible to process simultaneously a large number of samples, and the excellent recovery of RNA permits the use of this product for isolation of RNA from very small biological samples (biopsies, etc.).

IV. REAGENTS SUPPLIED

Ultraspec™ RNA is supplied in 50 ml, 100 ml, 200 ml, 500 ml, and 1 liter bottles.

V. STABILITY

Ultraspec™ RNA is stable for more than nine months when stored in the dark at 2-8°C.

VI. REAGENTS REQUIRED, BUT NOT SUPPLIED

Chloroform, isopropanol, 75% ethanol, and DEPC treated water (Ultraspec™ DEPC Treated Water, Biotecx, Cat. No. BL-5610).

VII. PROCEDURE

This method includes the following steps:

1. Homogenization Ultraspec™ RNA: 1 ml per 10-100mg tissue, or 5-10 x10⁶ cells
Note: A minimum of 1 ml of the reagent should be used for tissues <10 mg or cells <5 x 10⁶.
2. RNA Extraction 1 vol. of homogenate + 0.2 vol. of chloroform.
3. RNA Precipitation 1 vol. of aqueous phase + 1 vol. of isopropanol
4. RNA Wash 75% ethanol (2X)

Unless stated otherwise the procedure is carried out at 4°C.

CAUTION: IF THE Ultraspec™ RNA REAGENT IS SOLIDIFIED IN REFRIGERATOR, BRING SOLUTION TO ROOM TEMPERATURE, OR WARM THE REAGENT IN A 37°C WATER BATH (15-30 minutes) UNTIL THE REAGENT IS COMPLETELY IN SOLUTION.

THE REAGENT SEPARATES INTO TWO LAYERS. SHAKE THE BOTTLE THOROUGHLY EVERY TIME BEFORE USE, AND THE REAGENT WILL TURN MILKY WHITE.

FOR EXTRACTION OF RNA FROM MULTIPLE SAMPLES, SHAKE THE BOTTLE FREQUENTLY TO ENSURE HOMOGENEITY OF THE SOLUTION OR STIR THE REAGENT ON A MAGNETIC STIRRER WHILE REAGENT IS IN USE.

1. Homogenization

A. Tissues: Homogenize 10-100 mg of fresh tissue/ plant sample with 1 ml Ultraspec™ RNA reagent in hand-held glass-teflon or polytron homogenizer

NOTE: For best results, the frozen tissue or plant should be homogenized directly in the Ultraspec™ RNA solution.

B. Cells: Cells (eukaryotic/prokaryotic) grown in monolayer are lysed directly in a culture dish by adding the Ultraspec™ RNA (1 ml/3.5 cm petri dish) and passing the cell lysate several times through a pipette. The cell lysate should be transferred immediately into microfuge or polypropylene

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tubes. Cells grown in suspension are sedimented and then lysed in the Ultraspec™ RNA (1 mV5-10 x 10⁶ cells) by repetitive pipetting.

Note: Washing the cells before addition of the Ultraspec™ RNA should be avoided as this increases the possibility of mRNA degradation.

2. RNA Extraction

Following homogenization, store the homogenate for 5 minutes at 4° C to permit the complete dissociation of nucleoprotein complexes. Next, add 0.2 ml of chloroform per 1 ml of Ultraspec™ RNA, cover the samples tightly shake vigorously for 15 seconds and place on ice at 4° C for 5 minutes. Centrifuge the homogenate at 12,000 g (40 C) for 15 minutes.

After the addition of chloroform and centrifugation, the homogenate forms two phases: the lower organic phase and the upper aqueous phase. DNA and protein are in the organic phase and in the interphase while RNA is in the aqueous phase. The volume of the aqueous phase should be about 40-50% of the total volume of the homogenate plus chloroform.

3. RNA Precipitation

Carefully transfer the aqueous phase (4/5th volume) to a fresh tube while taking care not to disturb the interphase. Add equal volume of isopropanol and store samples for 10 minutes at 4° C. Centrifuge samples at 12,000g (40 C) for 10 minutes. RNA precipitate (often invisible before centrifugation) forms a white pellet at the bottom of the tube. In some cases RNA might precipitate along the sides of the tube. Care should be taken not to contaminate the side while washing the precipitate.

4. RNA Wash

Remove the supernatant and wash RNA pellet twice with 75% ethanol (1 ml of 75% ethanol/1 ml of reagent used) by vortexing and subsequent centrifugation for 5 minutes at 7,500g (4- C).

At the end of procedure, briefly dry the pellet under a vacuum for 5-10 minutes. It is important not to let the RNA pellet dry completely, as it will greatly decrease its solubility. However, as much ethanol should be removed as possible without completely drying the pellet. Dissolve the RNA pellet in 50-100ul of UltraspecDEPC treated water or in an appropriate buffer by vortexing for 1 minute (An incubation for 10-15 minutes at 55-60° C may be required to dissolve preparations of RNA).

NOTE: Do not use speed-vac for drying the pellet. DEPC treated RNase-free solution such as 1mM EDTA, pH 7.0 or 0.5% SDS solution can also be used for solubilizing of RNA.

VIII. YIELD AND PURITY OF RNA

The final preparation of undegraded RNA is free of DNA and proteins and has an A_{260}/A_{280} ratio of approximately 1.8-2.0.
Yield from 1 0 million mammalian cells = 100-200 pg
Yield from 100 mg tissue = 150-500 pg depending upon tissue

IX. NOTES AND COMMENTS

1. Following homogenization (before addition of chloroform) samples can be stored at -70°C for a longer period of time.
2. An additional precipitation may be necessary for enzymatic assays. Following solubilization, precipitate RNA in the presence of 1/10th vol. of 3M Sodium acetate with two volumes of ethanol for 15 minutes at 40 C. The PCR assays do not require an additional precipitation step.
3. 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.

X. SPECIAL HANDLING PRECAUTIONS

Ultraspec™ RNA reagent contains poison (phenol) and irritant (guanidine salts). CAN BE FATAL.

Use gloves and eye protection (shield, safety goggles).

Do not get in skin or clothing. Avoid breathing vapor. Also read the warning note on the bottle.

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

XI. REFERENCES

1. Sambrook, J. Fritsch, R. F., and Maniatis, R. *Molecular Cloning*. Cold Spring Harbor Laboratory Cold Spring Harbor N.Y. (1989).
2. Chirgwin, J. M., Przybyla, A.E., MacDonald, R. J. and Ruffer, W. J. *Biochemistry* 18,5294-5299 (1979).
3. Chomczynski, P and Sacchi, N. *Single-Step Method of RNA Isolation by Acid Guanidinium Thiocyanate- phenol-chloroform Extraction*. *Anal. Biochem.* 162, 156-159 (1987).
4. "CHAOSOLV, A concentrated Siopolymer Denaturant. *Biotech Bulletin #21*, 1992 (Biotech Laboratories, Inc.)

Polymerase Chain Reaction (PCR) technology is covered by U.S. Patents issued to Hoffmann-La Roche.

Cat No.	Size	Cat No.	Size
BL-10050	50ml	BL10500	500ml
BL-10100	100ml	BL11000	1L
BL-10200	200ml		

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