ULTRASPEC™-II RNA ISOLATION SYSTEM

For Laboratory use

BIOTECX BULLETIN NO: 28, 1993

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

I. INTENDED USE

For isolation of total RNA from tissues, cells, bacteria, plant, and yeast and purification by a specific RNA binding resin.

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™-II RNA Resin Purification System for total RNA isolation 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 and on the use of a specific RNA binding resin to purify total RNA. The Chaosolv in conjunction with phenol and other detergents is found to be very effective reagent for the isolation of total RNA from tissues and cells of human, animal, and bacterial origin. The RNA binding resin enhances the purification of total RNA and avoids conventional lengthy precipitation methods of ethanol and isopropanol.

In all single step isolation methods, the biological sample is homogenized with RNA extraction reagent and extracted with chloroform. The aqueous phase after centrifugation consists of RNA which is precipitated with ethanol or isopropanol. Ultraspec™-II RNA system purifies the RNA by mixing the aqueous phase with RNATack™ resin. The resin binds only the total RNA while other impurities are washed with 75% ethanol. The pure RNA is eluted from the resin with water or buffer. RNA thus prepared is free from impurities such as traces of guanidine salts, phenol, etc. which might interfere in subsequent RNA applications. Ultraspec™-II RNA system is highly reliable and produces very consistent results in 30–45 minutes.

III. APPLICATION

The total RNA isolated by this method is undegraded and free of proteins 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 RNA isolation using this system makes it possible to process simultaneously a large number of samples. This method can also isolate total RNA from very small biological samples (biopsies, etc.)

IV. REAGENTS SUPPLIED

The Ultraspec™-II RNA Kit contains the following:

1. Ultraspec™ RNA — 1 bottle of 50 ml, 100 ml, or 200 ml
2. RNATack™ Resin — 1 vial of 2 ml, 4 ml, or 8 ml

V. STABILITY

Ultraspec™-II RNA Resin Purification System Kit is stable for more than nine months when stored in the dark at 2–8° C.

VI. REAGENTS REQUIRED, BUT NOT SUPPLIED:

Chloroform, isopropanol and 75% ethanol. (These chemicals should be of high quality of minimum ACS grade); DEPC treated water (Biotecx, BL-5610)

VII. PROCEDURE:

This method includes the following steps:

1.	Homogenization	Ultraspec™ RNA (1 ml per 10–100 mg tissue, or 5-10 X 10^6 cells) **Note:** A minimum of 1 ml of the reagent should be used for tissues < 10 mg or cells < 5×10^6.
2.	RNA Extraction	1 vol. of homogenate + 0.2 vol. of chloroform
3.	RNA Purification	1 vol. of aqueous phase + 0.5 vol. of isopropanol + 0.05 vol. of RNA-Tack™ Resin. Wash resin pellet with 2X 1 ml 75% ethanol.
4.	RNA Elution	Elute RNA with 1 vol. (as that of resin) of DEPC treated water or buffer.

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

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 REGENT 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 reagent.

   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 tubes.

   NOTE: DO NOT LEAVE REAGENT IN CONTACT WITH POLYSTYRENE PLATES FOR MORE THAN A FEW MINUTES.

   Cells grown in suspension are sedimented and then lysed in Ultraspec™ RNA (1 ml/5–10 X 10^6 cells) by repetitive pipetting. 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 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 keep on ice (or at 4° C) for 5 minutes. Centrifuge the homogenate at 12,000 g (4° 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 proteins are in the organic phase and 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 Purification**

   Carefully transfer aqueous phase (4/5th volume) to a fresh tube while taking care not to disturb the interphase. Add 0.5 vol. of isopropanol and MIX. Add 0.05 vol. RNATack™ Resin (**NOTE:** MIX THE VIAL THOROUGHLY BEFORE USE) and vortex for 30 seconds.

   Spin for 1 minute in table top minicentrifuge. Discard the supernatant. Wash the pellet with 2X 1 ml of 75% ethanol by vortexing for 30 seconds and spinning for approximately 30 seconds. Discard the supernatant and briefly re-spin the tube and remove any traces of ethanol using the pipette tip. (Drying briefly for a few minutes in vacuum is recommended).

4. **RNA Elution**

   Resuspend pellet in 1 vol. (as that of resin) of DEPC Treated Water (Biotecx, BL-5610) or in an appropriate buffer (TE Buffer, Biotecx, BL-6530). Vortex for 30 seconds and spin for 1 minute. Transfer supernatant containing purified RNA to a new clean sterilized tube. Alternatively, the resuspended pellet can be transferred to a 0.45µ microspin column (Biotecx, BL-5909) and spun for 30–60 seconds to collect high quality 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 10 million mammalian cells = 100-200 µg

Yield from 100 mg tissue = 150–500 µg depending on 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. 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

The 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

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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 Biopolymer Denaturant,* Biotecx Bulletin #21, 1992 (Biotecx Laboratories. Inc.).

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

CAT. NO.	PRODUCT	SIZE
BL-12050	Ultraspec™ –II RNA	50 samples
BL-12100	Ultraspec™– II RNA	100 samples
BL-12200	Ultraspec™– II RNA	200 samples

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Revised August 1995

Printed in U.S.A.