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 six months).

3. REAGENTS REQUIRED, BUT NOT SUPPLIED

Chloroform (ACS grade)

Isopropanol (ACS grade)

75% Ethanol (ACS grade)

0.1% DEPC

4. METHOD

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

1. HOMOGENIZATION	RNAzol™ B (2 mL/100 mg tissue or 10 x 10^6 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.

Resuspend pellet in 0.5ml 1x PBS Cold

(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). Solubilize RNA by passing the lysate few times through the pipette.

10mls RNAzol B

50mls Cells Add 10mls RNAzol™

4.2 RNA EXTRACTION

1ml $CHCl_3$

Add 0.2 mL chloroform per 2 mL of homogenate, cover tightly the samples, shake vigorously for 15 seconds and let them stay on ice (or at 4 C) for 5 minutes. Centrifuge the suspension at 12,000 g (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 uper aqueous phase. RNA remains exclusively in the 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 tube.

2X Resuspend 1XTE

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.

0.5% SDS in TNE

At the end of procedure, dry briefly the pellet 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 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.

3 p/c/ethanol

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

5.3 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.

5.4 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.

5.5 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 also a warning note on the bottle.

REFERENCES

1. 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).

2. J. M. Chirgwin, A. E. Przybyla, R. J. MacDonald and W. J. Rutter, *Biochemistry* 18, 5294-5299 (1979).

3. *Molecular Cloning* (T. Maniatis, E. F. Fritsch and J. Sambrook, Eds.) pp. 188-209, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1982).

4. P. Chomczynski and N. Sacchi, *Anal. Biochem.* 162, 156-159 (1987).

RNAzol™	Catalog No.	CS-101	100 mL
		CS-102	200 mL
RNAzol™ B		CS-104 B	100 mL
		CS-105 B	200 mL
HETS™		CS-103	500 mL

HETS™ is a 15X concentrated solution of inorganic and organic salts designed to provide the highest efficiency of transfer of RNA from agarose gel to hybridization membranes and to visualize RNA on the membranes without staining with ethidium bromide. HETS™ cannot be used for transfer of RNA to unmodified nitrocellulose membranes.

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