MB601 RNA- XPress™ Reagent

Product Code

MB601-100 ml (100 ml RNA- XPress™ Reagent)
MB601-5 X100 ml (5X100 ml RNA- XPress™ Reagent)

Intended Use

Recommended for isolation of RNA from tissue, plant and bacteria.

Storage Conditions

RNA- XPress™ Reagent can be stored at room temperature (15-25°C) for upto 2 years without showing any reduction in performance.

Materials needed but not provided

- Chloroform (Product Code: MB109)
- Isopropyl alcohol (Product Code: MB063)
- 75% Ethanol (in DEPC-treated water)
- 0.5% SDS (Product code: ML007 – 20% SDS Stock solution).
- Molecular Biology Grade Water (RNase free) (Product code: ML024)

Introduction

RNA- XPress™ is a quick and convenient reagent to use in the isolation of RNA from human, animal, plant and bacterial samples. The protocol is rapid and permits isolation of RNA from large number of samples of small or large volumes. The RNA obtained can be further used for downstream applications such as Northern blot, mRNA isolation, *in vitro* translation, RNase and S1nuclease protection assay, RT-PCR and cloning. The procedure is very effective for isolating RNA molecules of all types from 0.1 to 15 kb in length.

Principle

HiMedia's RNA- XPress™ Reagent is designed for rapid purification of RNA from different samples. This product which is a mixture of guanidine thiocyanate and phenol in a mono-phase solution effectively dissolves RNA. After adding chloroform and centrifuging, the mixture separates into 3 phases: an aqueous phase containing the RNA, the interphase containing DNA and an organic phase containing protein. 1 ml of RNA-XPress™ Reagent is sufficient to isolate RNA from 50-100 mg of tissue, 5-10 X 10^6 cells or 10 cm^2 of culture dish surface, for cells grown in monolayer. This advanced RNA isolation procedure is an improvement to the single-step RNA isolation using phenol and guanidine isothiocyanate developed by Chomczynski and Sacchi. This is one of the most effective methods for isolating total RNA and can be completed in only 1 hour starting with fresh tissue and cells.

Concentration, yield & purity of RNA

The final preparation of RNA isolated with RNA- XPress™ Reagent is free of DNA and proteins. Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the RNA. Use RNase- Free Water to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm, and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should fall

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between 0.1 and 1.0. The 320 nm absorbance is used to correct for background absorbance. An absorbance of 1.0 at 260 nm corresponds to approximately 40 µg/ml of RNA. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. The A_{260}/A_{280} ratio should be ≥1.7. RNA purified by RNA- XPress™ Reagent is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions.

Concentration of RNA sample (µg/ml) = 40 x A_{260} x dilution factor.

Typical yields from tissues (µg RNA/mg tissue): Liver and spleen (4-10 µg), Kidney (2-4 µg), Skeletal muscle, brain (1-1.5 µg), Placenta (1-4 µg).

Typical yields from cultured cells (µg RNA/10^6 cells): Epithelial cells (6-15 µg), Fibroblasts (3-7 µg).

Precautions to be taken while handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even trace amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non-disposable vessels and solutions while working with RNA.

1. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible.

2. Use sterile, disposable plasticware and autoclavable pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipments.

3. Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1M NaOH and 1mM EDTA followed by RNase-Free Water. Alternatively, chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.

4. Glassware used for RNA work should be cleaned with a detergent, thoroughly rinsed, and oven baked at 240°C for four or more hours before use. Alternatively glassware can be treated with DEPC (Diethyl pyrocarbonate). Fill glassware with 0.1% DEPC treated water, allow to stand overnight at 37°C, and then autoclave or heat to 100°C for 15 minutes to eliminate residual DEPC.

5. Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS), thoroughly rinsed with RNase-Free Water, and then rinsed with ethanol and allowed to dry.

6. Solutions (water and other solutions) should be treated with 0.1% DEPC.

Specimen Handling and Collection

For Plant

Collect plant tissue in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage.

For tissues

Collect human/animal cells, tissues, blood sample in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage. Ensure that the tissue is at room temperature before beginning the protocol.

For bacteria

Collect overnight culture from sterile flask with the help of micropipette. Store the remaining culture at 2-8°C for short term use.

Types of Specimen

Tissue, plant, bacteria

Protocol

A. Sample Preparation

1. **Tissue**
   Homogenize tissue samples in RNA- XPress™ Reagent (1 ml for 50-100 mg of tissue) in a Homogenizer with serrated pestle S.P-2 ml (Product Code-GW117) or other appropriate homogenizer.

2. **Monolayer Cells**
   Lyse cells directly on the culture dish. Use 1 ml of RNA- XPress™ Reagent per 10 cm^2 of glass culture plate surface area. After addition of the reagent, the cell lysate should be mixed thoroughly using a micropipette to form a homogenous lysate.

   NOTE: RNA- XPress™ Reagent is not compatible with plastic culture plates.

3. **Suspension cells**
   Pellet up to 1 x 10^7 cells by centrifuging at 4°C for 5 minutes at 300 x g (≈1500 rpm) in a collection tube (not supplied) and then lyse in RNA- XPress™ Reagent by repeated pipetting. 1 ml of reagent is sufficient to lyse upto 10 X 10^6 animal, plant or cells or 10^7 bacterial cells.

NOTE:

I. Some bacterial cells may require a homogenizer.

II. After the cells have been homogenized or lysed in RNA- XPress™ Reagent, samples can be stored at –70°C for up to 1 month.

III. If samples have a high content of fat, protein, polysaccharides or extracellular material such as muscle, fat tissue and tuberous parts of plants, an additional step may be needed. After homogenization, centrifuge the homogenate at 12,000 x g (≈13,000 rpm) for 10 minutes at 4°C to remove the insoluble material (extracellular membranes, polysaccharides, and high molecular weight DNA). The supernatant contains RNA and Protein. If the sample had a high fat content there will be a layer of fatty material on the surface of the aqueous phase that should be removed. Transfer the clear supernatant to a fresh tube and proceed with step 2.

B. Phase separation

Incubate the homogenized samples for 5 minutes at room temperature (15-25°C) to permit the complete dissociation of nucleoprotein complexes. Add 200 µl of Chloroform per ml of RNA- XPress™ reagent used. Cover the sample tightly, shake vigorously for 15 seconds and allow to stand for 10 minutes at room temperature (15-25°C). Centrifuge the resulting mixture at 12,000 x g (≈13,000 rpm) for 15 minutes at 4°C. Following centrifugation, mixture separates into lower deep red

organic phase (containing protein), an interphase (containing DNA) and a colorless upper aqueous phase containing RNA.

NOTE: The chloroform used for phase separation should not contain Isoamyl alcohol and other additives.

C. RNA Precipitation

Transfer the aqueous phase containing RNA to a fresh tube and add 500 µl of Isopropyl alcohol. Allow the sample to stand for 5-10 minutes at room temperature (15-25°C). Centrifuge at 12,000 x g (≈13,000 rpm) for 10 minutes at 4°C. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.

D. RNA Wash

Remove the supernatant without disturbing the pellet and wash the RNA pellet by adding 1 ml (minimum) of 75% ethanol. Vortex the sample and then centrifuge at 7,500 x g (≈10,500 rpm) for 5 minutes at 4°C.

NOTE:

I. If the RNA pellets float, perform the wash in 75% ethanol at 12,000 x g (≈13,000 rpm).

II. Samples can be stored in ethanol for atleast 1 week at 4°C and upto 1 year at -20°C.

E. Redissolving the RNA

Discard the supernatant without disturbing the pellet. Briefly dry the RNA pellet for 5-10 minutes by air-drying or under a vaccum.

NOTE: Do not let the RNA pellet dry completely, as this will greatly decrease its solubility. Do not dry the RNA pellet by centrifugation under vacuum.

Add an appropriate volume (50µl) of RNase-Free Water to the RNA pellet. To facilitate dissolution, mix by repeated pipeting with a micropipette. Incubate at 55-60°C for 10-15 minutes.

Storage of the eluate with purified RNA: The eluate contains pure RNA, recommended to be stored at lower temperature (-80°C). Avoid repeated freezing and thawing of the sample which may cause denaturing of RNA.

NOTE: In rare occasions, when traces of DNA are observed, we recommend digesting the RNA with RNase-free DNase, if desired.

Warning and Precautions

Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

1. The yield of RNA depends upon the type and the volume of starting material used.

Performance and Evaluation

Performance of the kit is expected when the kit is used as per the protocol mentioned in the product insert within the expiry period when stored at recommended temperature.

Quality Control

Type of Sample	RNA Yield	RNA Purity
CHO cells	3-15 µg	1.8-2.1

Precautions and Disclaimer

HiMedia's RNA- Xpress™ Reagent is for laboratory use only, not for drug, household or other uses. This product which is a mixture of guanidine thiocyanate and phenol in a mono-phase solution effectively dissolves RNA. Take appropriate laboratory safety measures and wear gloves when handling. Avoid contact with skin, and use eye protection. In case of contact, wash with large amount of water. Seek medical attention. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

References

1. Chomczynski P., BioTechniques 15, 532-537(1993).
2. Chomczynski P. and Sacchi,N., Anal .Biochem.,162,156-159(1987)

Trouble Shooting Guide

Sr. No.	Problem	Sample	Cause	Solution
1.	Low yield of RNA	Any sample	Incomplete homogenization or lysis of samples	No particulate matter should remain in the tube. Incubate the homogenized samples for 5 minutes at room temperature (15-25°C) to permit the complete dissociation of nucleoprotein complexes.
			RNA pellet was not dissolved completely	To facilitate dissolution, mix by repeated pipetting with a micropipette at 55-60°C for 10-15 minutes.
2.	Degraded RNA	Any sample	The samples used for isolation or the isolated RNA preparations have been stored at -20°C	The samples should be stored at -80°C as specified in the procedure.
			Aqueous solution or tubes used for procedure may contain RNases	Use sterile, disposable plasticware and autoclavable pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipments.
3.	DNA contamination	Any sample	Presence of DNA	Samples should be digested with RNase- Free DNase.

Safety Information

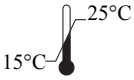
Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail to mb@himedialabs.com.



Storage temperature

Do not use if package is damaged

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