MB618PC16200 HiPurA® Pre-filled Cartridges for Tissue RNA Purification

Kit Contents

Product Code	Reagents provided	MB618PC16200
		48 PR
PF16J	Pre-filled Cartridges for Tissue RNA Extraction	48 no
LA1118B	Magnetic Rod Tip	12 no
DS0044	RNA- XPress™ Reagent	50 ml
DS0042	Elution Solution (RNase- Free Water)	4.5 ml
DS1005A	Magnetic Beads	1 ml

Intended use

Recommended for isolation of RNA from tissue samples.

Introduction

HiPurA® Pre-filled Cartridges for Tissue RNA Purification provides a fast and easy method for purification of total RNA for Northern analysis, Poly A^+ RNA selection, Primer extension, RNase and S1 nuclease protection assays, RT-PCR, Differential display, Expression-array and expression-chip analysis and cDNA library construction. The RNA purification procedure using magnetic beads comprises of three steps viz, adsorption of RNA to the magnetic beads, removal of residual contaminants and elution of pure RNA. The RNA obtained is compatible with various downstream applications as mentioned above.

HiPurA® Pre-filled Cartridges for Tissue RNA Purification

This kit simplifies isolation of RNA from tissues using magnetic bead-based procedure. The RNA – XPress™ Reagent provided in the kit helps in cell disruption and denaturation of tissue material. After adding Chloroform and centrifuging, the mixture separates into 3 phases: an aqueous phase containing the RNA, the interphase containing cell debris and DNA and an organic phase containing proteins. The aqueous phase, is then added to the binding solution followed by addition of magnetic beads in Pre-filled cartridge which promotes selective binding of RNA to the magnetic beads. After the initial binding of RNA, impurities like proteins, polysaccharides, low molecular weight metabolites and salts are removed by short washing steps. High quality RNA is finally eluted in the Elution Solution provided with the kit.

Elution

The yield of RNA depends on the sample type and the number of cells in the sample. A single elution with Elution Solution will provide sufficient RNA to carry out multiple amplification reaction.

Materials needed but not provided

- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- RNase – free pipette tips (aerosol barrier recommended)
- Mortar and pestle
- Liquid nitrogen
- Deoxyribonuclease I Solution (RNase-Free) and DNase Digest Buffer (Product Code: ML068)
- Nuclease-free 15 ml centrifuge tubes

ISO 9001-2015 CERTIFIED WHO GMP CERTIFIED ISO 13485-2016 CERTIFIED

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- Chloroform (Product Code: MB109)
- Insta NX® Mag16 (LA1118)
- Cartridge Holder (LA1118CH)
- Insta NX® Mag32 (LA1096)
- HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017)

Storage

HiPurA® Pre-filled Cartridges for Tissue RNA Purification can be stored at room temperature (15-25°C) for up to 2 years without showing any reduction in performance. We advise a certain storage temperature for the reagents listed below:

- **On receipt store Magnetic Beads (DS1005A): at 2-8°C.**

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 minute 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 equipment's.

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, 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 (0.1% in 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.

General Preparation Instructions

1. **Thoroughly mix reagents**
   Examine the reagents for precipitation. If any kit reagent forms a precipitate (other than enzymes), warm at 55-65°C until the precipitate dissolves and allow cooling to room temperature (15-25°C) before use.

2. Prechill the mortar and pestle to -20°C before use.

3. Ensure that clean & dry DNase and RNase free tubes and tips are used for the procedure.

4. Set the microcentrifuge at 4°C before starting the protocol for step 3.

Specimen Handling and Collection

Collect tissue 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.

Types of Specimen

Sample: tissue

Sample Preparation

Finely cut the tissue material before grinding. Weigh 30 mg of the tissue and grind properly using a mortar and pestle in liquid nitrogen to a fine powder. Allow the liquid nitrogen to evaporate. DO NOT ALLOW THE SAMPLE TO THAW (keep samples on ice if needed). Proceed immediately to the RNA isolation protocol.

NOTE: If there is no information about the nature of the starting material, it is recommended to start with no more than 50 mg of tissue or 3-4 x 10^6 cells. It may be possible to use up to 100 mg tissue material or up to 1 x 10^7 cells in subsequent preparations, depending on RNA yield and purity. Counting cells or weighing tissue is the most accurate way to quantitate the amount of starting material.

NOTE: Delay in continuing to RNA isolation after sample preparation will result in RNA degradation and yield loss.

Procedure

1. To the ground tissue material, immediately add **1 ml of RNA- XPress™ Reagent (DS0044)** and mix thoroughly. **(Do not grind the tissue material after the addition of RNA- XPress™ Reagent, as it will cause shearing of RNA).**

2. Transfer the mixture to a 2.0 ml capped collection tube (not provided).

3. **Phase separation**
   Incubate the sample 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 5-10 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 organic phase (containing protein), an interphase (containing cell debris and DNA) and upper aqueous phase containing RNA. Collect the upper layer separately. **This will be your pre-processed sample**

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

   Optional DNase Treatment:

- Add 10 µl of DNase I Solution (not provided) to 70 µl of DNase Digest Buffer. Mix by inversion. Do not vortex.

- Add 80 µl of DNase I/Digest Buffer (not provided) mixture directly to the pre-processed sample. Incubate at room temperature for 5 minutes.

Set up processing cartridge as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Select **"MBMAG618"** program.
3. Open the door of Insta NX® Mag16 machine.
4. Place the Pre-filled Cartridges for Tissue RNA Extraction (PF16J) into the Cartridge Holder (LA1118CH). Remove the seal from the Pre-filled Cartridges for Tissue RNA Extraction (PF16J).

   NOTE: Take care while peeling off the seal. Hold the cartridge firmly by one hand and then peel off the seal by holding it in your other hand without shaking the cartridge.
5. Add **50µl of Elution Solution (RNase- Free Water) (DS0042)** in the **6th well** of **the Pre- filled Cartridges for Tissue RNA Purification (PF16J).**
6. Add **450µl pre- processed sample in the 1st well of the** Pre-filled Cartridges for Tissue RNA Extraction **(PF16J).**
7. **Add 20µl Magnetic Beads (DS1005A) in the 1st well of the** Pre-filled Cartridges for Tissue RNA Extraction **(PF16J).**
8. After adding the above solutions place the cartridges along with the cartridge holder on the platform.
9. Place the Magnetic Rod Tip (LA1118B) by sliding onto the machine.

   NOTE: After placing the rods ensure that the rods are properly fixed on their place.
10. Click on the **RUN** option on the home screen.
11. After the run is complete, remove Pre-filled Cartridges for Tissue RNA Extraction (PF16J) from the position. Discard the Magnetic Rod Tip (LA1118B). Dispense the eluted DNA from well 6 to a new HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017) (not provided). The eluate contains pure DNA.

    NOTE: If small amount of magnetic beads are observed in the final eluate then keep the cartridges along with cartridge holder on Magnetic pad (not provided) for 4-5 minute and collect supernatant carefully without disturbing beads pellet in new collection tube.

    OR

    Take out eluate in new collection tube and centrifuge at higher speed for around 1 min to pellet down the traces of Magnetic beads present in the eluate.

*NOTE: If you process less than 4 samples at a time please order LA1118B- Magnetic Rod Tip (Pack size- LA1118B-4no/ LA1118B-40no).

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.

References

1. Sambrook, J., *et al*. Molecular Cloning: A laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1989)
2. Farrell, Robert E., Jr.; RNA Methodologies; 2nd Edition; Academic Press: NY, 1998; pp.37-53(Cat. No. Z350354)

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 laboratory practices while handling samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

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

Performance and Evaluation

The yield and efficiency of purification is determined by performing Real- Time PCR.

Quality Control

Each lot of HiMedia's HiPurA® Pre-filled Cartridges for Tissue RNA Purification is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Pre-filled Cartridges for Tissue RNA Purification is for laboratory use only, not for drug, household or other uses. 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 of 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.

Please refer disclaimer Overleaf.

Symbols

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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