MB603MPF16 HiPurA® Pre-filled Plate for Plant RNA Purification

Kit Contents

Product Code	Reagents provided	MB603MPF16
		96PR
PF16R	Pre-filled Plate for plant RNA Extraction	6 no
LA1118B	Magnetic Rod Tip	12 no
DS0044	RNA- XPress™ Reagent	102 ml
DS0078	Binding Solution (PBR)	102 ml
DS0042	Elution Solution (RNase- Free Water)	7 ml
DS1005A	Magnetic Beads	2.1 ml

Intended use

Recommended for isolation of RNA from Plant samples.

Introduction

HiPurA® Pre-filled Plate for Plant 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 Plate for Plant RNA Purification

This kit simplifies isolation of RNA from Plants using magnetic bead-based procedure. The RNA – XPress™ Reagent provided in the kit helps in cell disruption and denaturation of Plant 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 Plate 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.

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

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Storage

HiPurA® Pre-filled Plate for Plant 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**

Materials needed but not provided

- Tabletop Microcentrifuge at 4°C (with rotor for 2.0 ml tubes)
- RNase – free pipette tips (aerosol barrier recommended)
- Mortar and pestle
- Liquid nitrogen
- Chloroform (Product Code: MB109)
- HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017)
- Polypropylene sealing film (Product Code: PR21)
- Insta NX® Mag16 (LA1118)

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. **ß -mercaptoethanol (ß -ME) must be added to Binding Solution (PBR).**
   ß -ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10µl ß –ME per 1ml Binding Solution (PBR) (DS0078). Binding Solution (PBR) (DS0078) containing ß-ME can be stored at room temperature for up to 1 month.

2. **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.

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

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

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

Sample Preparation

Finely cut the leaf material before grinding. Midrib should be removed before cutting the leaves, as midrib is a major source of carbohydrate contamination. Weigh 100 mg of the finely cut plant material 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 plant material or 3-4 x 10^6 cells. It may be possible to use up to 100 mg plant material or up to 1 x 10^7 cells in subsequent preparations, depending on RNA yield and purity. Counting cells or weighing Plant 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 plant material, immediately add 1 ml of RNA- XPress™ Reagent (DS0044) and mix thoroughly **(Do not grind the plant 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 (not provided) per ml of RNA- XPress™ Reagent (DS0044) 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.

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

4. Transfer the aqueous phase containing RNA to a fresh 2.0 ml capped collection tube (not provided) and add 1 ml of Binding Solution (PBR) (DS0078). Mix thoroughly by gentle pipetting. **This will be your pre-processed sample**

Set up processing cartridge as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Select **"MB603M"** program.
3. Open the door of Insta NX® Mag16 machine.
4. Remove the seal from the Pre-filled Plates for Plant RNA Extraction (PF16R).

   NOTE: Take care while peeling off the seal. Hold the plate firmly by your left hand and then peel off the seal by holding it in your right hand without shaking the plate.

5. Add **50µl of Elution Solution (RNase-free water) (DS0042)** into the **6th and 12th column of the Pre-filled Plates for Plant RNA Extraction (PF16R).**
6. Add approximately **350µl pre- processed sample in the 1st and 2nd column** and for another sample add approximately **350µl pre- processed sample in 7th and 8th column of the Pre-filled Plates for Plant RNA Extraction (PF16R).**

   NOTE: Column 1st, 2nd of **the Pre-filled Plates for Plant RNA Extraction (PF16R)** should contain same sample material. Similarly, column 7th, 8th of the **Pre-filled Plates for Plant RNA Extraction (PF16R)** should contain same sample material.

7. **Add 10µl Magnetic Beads (DS1005A) in the 1st & 2nd column and 7th & 8th column of the Pre-filled Plates for Plant RNA Extraction (PF16R).**
8. After adding the above solutions place the plate on the platform of the machine.

   NOTE: 16 samples can be processed in a single Pre-filled Plate for Plant RNA Extraction (PF16R).

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. Closed the door and Click on the **RUN** option on the home screen.
11. After the run is complete discard the Magnetic Rod Tip (LA1118B). Remove the Pre-filled Plate for Plant RNA Extraction (PF16R) from the position. Dispense the eluted nucleic acid from column 6 and column 12 to a new HiPer® Lock Microcentrifuge Tube, 2.0ml (MBLA1017) (not provided). The eluate contains pure nucleic acid.

    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.

    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 Plate for Plant RNA Purification is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Pre-filled Plate for Plant 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

Identification No.: PIMB603MPF16
Rev. No.: 01
Date of Issue: 2025-04

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