MB603 HiPurA® Plant and Fungal RNA Miniprep Purification Kit

Kit Contents

Product Code	Reagents provided	MB603		
		20 Preps	50 Preps	250 Preps
DS0044	RNA - XPress™ Reagent	24 ml	60 ml	300 ml
DS0078	Binding Solution (PBR)	24 ml	60 ml	300 ml
DS0041	Prewash Solution (RW1)	20 ml	50 ml	250 ml
DS0012	Wash Solution Concentrate (WS)	8 ml	20 ml	100 ml
DS0042	Elution Solution (RNase- Free Water)	2.4 ml	6 ml	30 ml
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20nos	50 nos	250 nos
DBCA016	Collection Tube (Uncapped), Polypropylene (2.0 ml)	20 nos	50 nos	250 nos
PW1139	Collection Tube, Polypropylene (2.0 ml)	40 nos	100 nos	2 X 250 nos

Introduction

HiPurA® Plant and Fungal RNA Miniprep Purification Kit 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 the miniprep spin columns comprises of three steps viz, adsorption of RNA to the membrane, removal of residual contaminants and elution of pure RNA. HiMedia's HiElute Miniprep Spin Column (Capped) format allows rapid processing of multiple samples. The columns have a high binding capacity and high quality RNA is obtained from various species. The RNA obtained is compatible with various downstream applications as mentioned above.

HiPurA® Plant and Fungal RNA Miniprep Purification Kit

This kit simplifies isolation of RNA from plant cells, tissues and filamentous fungi with spin-column procedure. The RNA – XPress™ Reagent provided in the kit helps in cell disruption and denaturation of the 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. To the aqueous phase, Binding Solution (PBR) and ethanol are added, which promote selective binding of RNA to the HiElute Miniprep Spin Column (Capped) membrane. 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. Typical yield is 15-65 µg of total RNA from 100 mg of the plant material, depending on the type of tissue and plant species.

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

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HiElute Miniprep Spin Column (Capped) [DBCA03]

HiElute Miniprep Spin Column (capped) is based on the advanced silica binding principle presented in a microspin format. The system efficiently couples the reversible nucleic acid-binding properties of the advanced silica gel membrane and the speed plus versatility of spin column technology to yield high quantity of RNA. The use of spin column facilitates the binding, washing, and elution steps thus enabling multiple samples to be processed simultaneously. RNA binds specifically to the advanced silica-gel membrane while contaminants pass through. PCR inhibitors such as divalent cations and proteins are completely removed in two efficient wash steps leaving pure nucleic acid to be eluted in the Elution Solution provided with the kit. The ratio of 28S rRNA to 18S rRNA should be 2:1. The ribosomal RNAs should appear as sharp bands or peaks.

Elution

The yield of RNA depends on the sample type and the number of cells in the sample. A single elution with 30-50 µl of Elution Solution will provide sufficient RNA to carry out multiple amplification reaction.

Concentration, yield and purity of RNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the RNA. Use Elution Solution or 10mM Tris-Cl, pH 8.5 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 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. The A_{260} - A_{320} /A_{280}-A_{320} ratio should be 1.8–2.1. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. RNA purified by HiPurA® Plant and Fungal RNA Miniprep Purification Kit 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.

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
- Ethanol (96-100%)
- 2- mercaptoethanol (ß -ME) (Product Code: MB041)
- Deoxyribonuclease I Solution (RNase-Free) and DNase Digest Buffer (procure from any standard company)
- Nuclease-free 15 ml centrifuge tubes
- Chloroform (Product Code: MB109)

Storage

HiPurA® Plant and Fungal RNA Miniprep Purification Kit can be stored at room temperature (15-25°C) for upto 1 year without showing any reduction in performance.

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 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, 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). Binding Solution (PBR) 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. **Dilute Wash Solution Concentrate (WS) (DS0012) as follows:**

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100 %)
20	8 ml	24 ml
50	20 ml	60 ml
250	100 ml	300 ml

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

Centrifugation

All centrifugation steps are carried out in conventional laboratory centrifuge e.g. Beckman CS-6KR, Heraeus Varifuge 3.0R, or Sigma 6k10 with fixed angle rotor. The tubes provided with the kit are compatible with almost all laboratory centrifuges and rotors. All centrifugation steps are given in g, the correct rpm can be calculated using the formula:

$$RPM = \sqrt{RCF/1.118 \times 10^{-5} r}$$

Where RCF = required gravitational acceleration (relative centrifugal force in units of g); r = radius of the rotor in cm; and RPM = the number of revolutions per minute required to achieve the necessary g-force

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. Do not overload the HiElute Miniprep Spin Column (Capped), as this will significantly reduce RNA yield and quality. 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 plant material, immediately add 1 ml of RNA- XPress™ Reagent 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.

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.

   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 tube (not provided) and add 1 ml of Binding Solution (PBR) (DS0078). Mix thoroughly by gentle pipetting. Transfer the entire solution to a 15 ml tube (not provided).

5. Add 0.5 volumes (usually 775 µl) of ethanol (96-100%) to the above solution, and mix immediately by gentle pipetting. Do not centrifuge. Continue without delay with step 6.

6. **Load Lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Apply sample (usually 700 µl), including any precipitate that may have formed, on the HiElute Miniprep Spin Column (Capped) placed in a 2 ml uncapped collection tube. Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≈10,000 rpm). Discard the flow-through.

   NOTE: Since the volume exceeds 700 µl, load aliquots successively onto the HiElute Miniprep Spin column and centrifuge as above. Discard the flow-through after each centrifugation step.

7. **Optional: On Column DNase I digestion**
   Generally, DNase I digestion is not required since the solutions of this kit efficiently removes almost all the DNA without DNase treatment. However, further DNase treatment may be necessary for certain RNA applications that are sensitive to small amounts of DNA (e.g.TaqMan RT-PCR analysis with a low abundant target). DNA can also be removed by DNase digestion.

   Carryout lysis, homogenization, and loading onto the HiElute Miniprep Spin Column as indicated above. Instead of continuing with the Prewash Solution (RW1) in step 8, follow steps a –d below.

a. Add 350 µl of Prewash Solution (RW1) into the HiElute Miniprep Spin column, and centrifuge for 1 minute at ≥8000 x g (≈10,000 rpm). Discard the flow through and reuse the collection tube in step 6c.

b. Add 10 µl of DNase I Solution to 70 µl of DNase Digest Buffer. Mix by inversion. Do not vortex.

c. Add 80 µl of DNase I/Digest Buffer mixture directly onto the HiElute Miniprep Spin Column. Incubate at room temperature for 15 minutes.

d. Add 350 µl of Prewash Solution (RW1) into the HiElute Miniprep Spin Column, and centrifuge for 1 minute at ≥8000 x g (≈10,000 rpm). Discard the flow through and continue with the step 8.

OR

Alternatively, residual DNA can be removed by a DNase I digestion after RNA isolation.

8. Add 700 µl of Prewash Solution (RW1) (DS0041) to HiElute Miniprep Spin Column. Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≈10,000 rpm). Discard the flow through.

9. Add 500 µl of diluted Wash Solution (WS) (DS0012). Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≈10,000 rpm) to wash the column. Discard the flow-through.

   NOTE: **Wash Solution (WS) is supplied as a concentrate. Ensure that ethanol is added to Wash Solution Concentrate (WS), as indicated in General Preparation Instructions.**

10. Add another 500 µl of diluted Wash Solution (WS) to the HiElute Miniprep Spin Column. Close the tube gently, and centrifuge for 2 minutes at ≥8000 x g (≈10,000 rpm) to dry the membrane. Spin the empty column for additional 1minute at ≥13,000 x g (≈14,000 rpm) for drying of the column as it will avoid Wash Solution carryover.

11. **RNA Elution**
    Transfer the column to a new uncapped 2 ml collection tube. Add 30-50 µl Elution Solution (RNase-Free Water) directly onto the spin column. Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≈10,000 rpm) to elute.

12. If the expected RNA yield is >20 µg, repeat the elution step (step 10) as described, with a second volume of RNase-Free Water. Elute into the same collection tube. Transfer the eluate into fresh capped 2.0ml collection tube for longer storage.

    NOTE: To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate. The yield will be 15-30% less than the yield obtained using a second volume of RNase-Free Water, but the final concentration will be higher.

13. 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)

Precautions

Read the procedure carefully before starting the experiment.

Performance and Evaluation

Each lot of HiMedia's HiPurA® Plant and Fungal RNA Isolation Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Yield	DNA Purity
Plant leaf	15-65 µg	1.8-2.1

Trouble shooting Guide

Sr. No.	Problem	Possible Cause	Solution
1.	Clogged HiElute Miniprep Spin Column (Capped)	Inefficient disruption and /or homogenization	See sample preparation.
		Amount of starting material used is more than recommended	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material.
2.	Low RNA yield	RNA still bound to the membrane	Repeat elution, by incubating the HiElute Miniprep Spin Column (Capped) for 10 minutes at room temperature with RNase-Free water before centrifugation.

		Ethanol carry over	During the second wash with Buffer (WS), the membrane of the column should be dried by spinning at ≥8000 x g (≈10,000 rpm) for 2 minutes at 15-25°C. Following centrifugation remove the column from the centrifuge tube carefully so that the column does not contact the flow-through as this will result in carryover of ethanol. To eliminate any chance of ethanol carryover, transfer the column to a new 2 ml collection tube and perform the optional 1 minute centrifugation step as mentioned in the protocol.
		Low A_{260}/A_{280} value	Use 10mM Tris-Cl, pH 8.5, and not RNase-Free water, to dilute the samples before measuring purity.
3.	RNA Degraded	RNase contamination	The reagents provided in the kit have been tested and guaranteed RNase-free. RNases can be introduced during use. Ensure that RNases are not introduced during the procedure or later handling.
4.	RNA does not perform well in downstream experiments	Salt carry over during elution	Ensure that Wash Solution (WS) is at 15-25°C. When reusing collection tubes between washing steps, remove residual flow-through from the rim by blotting on clean paper towels.
		Ethanol carry over	During the second wash with Wash Solution (WS), the membrane of the column should be dried by spinning at ≥8000 x g (≥10,000 rpm) for 2 minutes at 15-25°C. Following centrifugation remove the column from the centrifuge tube carefully so that the column does not contact the flow-through as this will result in carryover of ethanol. To eliminate any chance of ethanol carryover, transfer the column to a new 2 ml collection tube and perform the optional 1 minute centrifugation step as mentioned in the protocol.

5.	DNA contamination in downstream experiments	No incubation with Prewash Solution (RW1)	In subsequent preparations, incubate the column for 5 minutes at room temperature after the addition of Prewash Solution (RW1) before centrifugation.
		No DNase treatment	Follow the optional on-column DNase digestion using RNase-Free DNase indicated in the protocol (step no. 6a-6d).

Safety Information

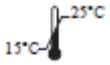
The HiPurA® Plant and Fungal RNA Miniprep Purification Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) 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 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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