MB611 HiPurA® Yeast RNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB611		
		20 preps	50 preps	250 preps
DS0037	RNA Lysis Solution (HRL)	10 ml	25 ml	125 ml
DS0041	Prewash Solution (RW1)	30 ml	75 ml	375 ml
DS0012	Wash Solution Concentrate (WS)	6 ml	15 ml	75 ml
DS0042	Elution Solution (RNase- Free Water)	2 ml	5 ml	25 ml
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20 no.	50 no.	250 no.
DSCA02	HiShredder (in DBCA016 Collection Tube)	20 no.	50 no.	250 no.
DBCA016	Collection Tube (Uncapped), Polypropylene (2.0 ml)	20 no.	50 no.	250 no.
DBCA017	Collection Tube, Polypropylene (2.0 ml)	40 no.	100 no.	2 x 250 no.

Introduction

HiPurA® Yeast RNA Purification Kit provide 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® Yeast RNA Purification Kit

This kit simplifies isolation of yeast RNA with spin-column procedure. The lysis buffer provided in the kit helps in cell disruption and denaturation, samples are centrifuged through a HiShredder which removes insoluble material and reduces the viscosity of the lysate by disrupting viscous material. Ethanol is added to the cleared lysate, which promotes 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. This kit yields up to 90 µg of total RNA from 30 million yeast cells.

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 gel membrane and the speed plus versatility of spin column technology to yield high quantity of RNA.

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

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The use of spin column facilitates the binding, washing, and elution steps thus enabling multiple samples to be processed simultaneously. This column eliminates the need for alcohol precipitation, expensive resins, and harmful organic compounds such as phenol and chloroform, otherwise employed in traditional RNA isolation techniques. 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 RNA 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 to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260nm, 280nm, and 320nm using a quartz microcuvette. Absorbance readings at 260nm should fall between 0.1 and 1.0. The 320nm absorbance is used to correct for background absorbance. An absorbance of 1.0 at 260nm 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 260nm to absorbance at 280nm. RNA purified by HiPurA® Yeast RNA 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

- 30°C water bath
- Tabletop Microcentrifuge (with rotor for 2.0ml tubes)
- RNase - free pipette tips (aerosol barrier recommended)
- Ethanol (70%)
- 2- mercaptoethanol (ß -ME) (Product Code: MB041)
- Deoxyribonuclease I Solution (RNase-Free) and DNase Digest Buffer (procure from any standard company)
- Lyticase/zymolase, 1M Sorbitol and 0.1 M EDTA, pH 7.4 - For Isolation of total RNA from Yeast

Storage

HiPurA® Yeast RNA Purification Kit can be stored at room temperature (15-25°C) for up to 18 months 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 automatic 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 min 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 RNA Lysis Solution (HRL) before use.**
   ß-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10µl ß-ME per 1ml Lysis Solution. Lysis Solution containing ß-ME can be stored at room temperature (15-25°C) 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. Ensure that clean & dry DNase, RNase free tubes and tips are used for the procedure.

4. **Dilute Wash Solution Concentrate (WS) (DS0012) as follows:**

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100 %)
20	6 ml	18 ml
50	15 ml	45 ml
250	75 ml	225 ml

1. **Prepare Sorbitol buffer as follows:**
   1M sorbitol
   100 mM EDTA

   Just before use, add:
   10 µl of ß-mercaptoethanol per 1ml of Sorbitol Buffer.

2. Preset the centrifuge at 4°C for step 1 and 2.

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 performed at room temperature (15-25°C) and 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.

Procedure

1. Grow yeast culture *Saccharomyces cerevisiae* or *Candida spp.* in YPD medium (Product Code: M1363). Harvest cells, maximum up to 1 X 10^8 or up to 1.5ml of overnight grown yeast culture in capped 2ml centrifuge tube by centrifuging at 1500 rpm for 5 minutes at 4°C. Remove the culture medium completely and discard.

2. **Resuspend cells**
   Resuspend the pellet in 600 µl of Sorbitol Buffer (Refer General Preparation Instructions). Add 50U of zymolyase or lyticase and incubate at 30°C for 30 minutes.

3. Pellet the spheroplasts by centrifuging for 10 minutes at 6500 x g (10,000 rpm) at 4°C. Discard the supernatant without disturbing the pellet

4. **Lysis reaction**
   Add 350 µl of RNA Lysis Solution (HRL) (DS0037) to the pellet. Vortex or pipet to mix.

   NOTE: If insoluble material is visible, centrifuge for 2 minutes at maximum speed, and use only the supernatant in the subsequent steps.

   NOTE: Ensure that ß -ME is added to RNA Lysis Solution (HRL) before use.

5. Pipet the lysate directly into a HiShredder (DSCA01) placed in a 2ml collection tube, and centrifuge for 2 minutes at maximum speed. Continue with step 5.

6. **Prepare for binding:**
   Add 1 volume of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not centrifuge.

   NOTE: A precipitate may be visible after the addition of ethanol. This does not affect the procedure.

7. **Load Lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Apply sample including any precipitate that may have formed, on the HiElute Miniprep Spin Column. Close the tube gently, and centrifuge for a minute at ≥8000 x g (≥10,000 rpm) at room temperature (15-25°C). Discard the flow-through.

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

   Optional: On Column DNase digestion
   Generally, DNase digestion is not required since the solutions of this kit efficiently remove most of 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 column as indicated above. Instead of continuing with the Prewash Solution (RW1) in step 7, follow steps a –d below.

a. Pipet 350 µl of Prewash Solution (RW1) into the column, and centrifuge for a minute at ≥8000 x g (≥10,000 rpm) at room temperature (15-25°C). Discard the flow through and reuse the collection tube in step c.

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 column. Incubate at room temperature (15-25°C) for 15 minutes.

d. Pipet 350 µl of Prewash Solution (RW1) into the column, and centrifuge for a minute at ≥8000 x g (≥10,000 rpm) at room temperature (15-25°C). Discard the flow-through and continue with the step 8.

OR

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

8. Add 700 µl of Prewash Solution (RW1) (DS0041) to the column centrifuge at ≥8,000 x g (≥10,000 rpm) at room temperature (15-25°C) for 1 minute. Discard the flow-through. Reuse the collection tube in step 9.

9. Transfer the column into a 2ml collection tube. Pipet 500 µl of diluted Wash Solution (WS) (DS0012). Close the tube gently, and centrifuge for a minute at ≥8000 x g (≥10,000 rpm) at room temperature (15-25°C) 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).

10. Add another 500 µl of diluted Wash Solution (WS) to the column. Close the tube gently, and centrifuge for 2 minutes at ≥8000 x g (≥10,000 rpm) at room temperature (15-25°C) to dry the membrane. Continue directly with step 10, or to eliminate any chance of possible Wash Solution carryover, continue with step 9a.

    Optional: Place the column in a new 2ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge in a microcentrifuge at maximum speed for 1 minute.

11. **RNA Elution**

    Transfer the column to a new uncapped 2ml collection tube. Pipet 30-50 µl Elution Solution (RNase-Free Water) directly onto the column. Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) at room temperature (15-25°C) to elute.

12. If the expected RNA yield is >30 µg, repeat the elution step (step 10) as described with a second volume of RNase-Free Water. Elute into the same collection tube.

13. Transfer the eluate into 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.

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; pp. 7.3-7.5)
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® Yeast RNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Yield	DNA Purity
S. cerevisiae	Upto 90 µg	1.8-2.1

Trouble shooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Clogged HiElute Miniprep Spin Column	Too much of starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material.
		Centrifugation temperature is too low	The centrifugation temperature should be 20 – 25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the HiElute Miniprep Spin Column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol containing lysate to 37°C before transferring it to the HiElute Miniprep Spin Column.
2.	Low RNA Yield	Too much of starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material.
		RNA still bound to column	Repeat RNA elution, but incubate the column for 10 minutes at room temperature (15-25°C) with Elution Solution (RNase free water) before centrifuging.

		Ethanol carryover	During the second wash with Wash Solution (WS), be sure to centrifuge for 2 minutes at ≥8000 x g (≥10,000 rpm) at room temperature (15-25°C) to dry the column. After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow through otherwise carryover of ethanol will occur. To eliminate any chance of possible ethanol, centrifuge the column for another minute at maximum speed.
3.	Low A_{260}/A_{280}	Water used to dilute RNA for A_{260}/A_{280} measurement	Use 10 mM Tris – Cl, pH 7.5, not RNase free water to dilute the sample before measuring purity.
4.	DNA contamination in downstream experiments	No incubation with Prewash Solution (RW1)	In subsequent preparations, incubate the column for 5 minutes at room temperature (15-25°C) after the addition of Prewash Solution (RW1) before centrifuging.
		No DNase treatment	Follow the optional on-column DNase digestion step.
5.	RNA does not perform well in downstream experiments	Ethanol carryover	During the second Wash using Wash Solution (WS), be sure to dry the column membrane by centrifugation at ≥8000 x g (≥10,000 rpm) at room temperature (15-25°C) for 2 minutes to dry the membrane. Following the centrifugation, remove the column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

Safety Information

The HiPurA® Yeast RNA Purification Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves while 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.

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.: PIMB611
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