# MB613 HiPurA® Bacterial RNA Purification Kit

**Kit Contents**

| Product Code | Reagents provided | MB613 | | |
|---|---|---|---|---|
| | | 20 Preps | 50 preps | 250 preps |
| MB098 | Lysozyme | 80 mg | 200 mg | 1 g |
| DS0037 | RNA Lysis Solution (HRL) | 14 ml | 35 ml | 175 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. |
| PW1139 | Collection Tube, Polypropylene (2.0 ml) | 40 no. | 100 no. | 2 x 250 no. |

**Introduction**

HiPurA® Bacterial RNA Purification Kit provides a fast and easy method for purification of Bacterial 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® Bacterial RNA Purification Kit**

This kit simplifies isolation of RNA from different types of bacteria (Gram positive and Gram negative) 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 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.



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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 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. 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 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 purified**

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 320 nm using a quartz microcuvette. Absorbance readings at 260nm should fall between 0.1 and 1.0. The 320nm absorbance is used to correct 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® Bacterial 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**

- 37°C water bath or heating block
- Tabletop Microcentrifuge (with rotor for 2.0ml tubes)
- RNase – free pipette tips (aerosol barrier recommended)
- Ethanol (96 - 100%)
- 2- mercaptoethanol (ß -ME) (Product Code: MB041)
- Deoxyribonuclease I Solution (RNase-Free) and DNase Digest Buffer (procure from any standard company)
- TE Buffer (10 mM Tris Cl, 1mM EDTA pH 8.0) (Product Code: ML012/ML060)

**Storage**

HiPurA® Bacterial RNA Purification Kit can be stored at room temperature (15-25°C) for up to 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 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. **β-mercaptoethanol (β-ME) must be added to RNA Lysis Solution (HRL) (DS0037) before use.**
   β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10µl β-ME per 1ml Lysis Solution. Lysis Solution containing β-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 |

5. **Prepare Lysozyme Solution (Product Code: MB098)**
   Prepare a **20 mg/ml** solution of Lysozyme (approximately 2.115 x $10^6$ unit/ml) in TE Buffer (10mM Tris Cl, 1mM EDTA pH 8.0) **(not provided)**. Lysozyme solution should be freshly prepared prior to use.

Example: In order to make 1ml of Lysozyme solution, dissolve 20mg of lysozyme (provided) in 1ml of TE Buffer (10mM Tris Cl, 1mM EDTA pH 8.0) (not provided). Pipette the mixture up and down or vortex to dissolve the lysozyme.

**NOTE:** Lysozyme dissolves readily by pipetting up and down as opposed to vortexing. Excessive vortexing may cause foaming.
For each DNA preparation, 100 µl of lysozyme solution is required. Make extra solution to account for pipetting error. The lysozyme solution should be preferably used on the day of preparation. If some Lysozyme stock solution is left, it can be stored at -20°C.

6. **Bacterial cells should be harvested in log-phase of growth.**
(Inoculate a single colony in 30-50ml broth and incubate for 3 hours at desired temperature)

**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.

**Important notes before starting**

For bacterial cultures containing high levels of RNA, fewer bacteria should be used in order not to exceed the RNA binding capacity of the HiElute Miniprep Spin Column (100 µg RNA). For bacterial cultures containing lower levels of RNA, the maximum number of bacteria can be used. For RNA isolation, bacteria should be harvested in log-phase growth, do not use overnight culture.

Prepare TE Buffer, pH 8.0, with 20 mg/ml lysozyme for either Gram-negative bacteria or for Gram-positive bacteria.

The bacterial culture should be harvested at 4°C. All subsequent steps of the protocol should be performed at room temperature (15-25°C).

**Procedure**

**For Gram Negative and Gram Positive Bacteria:**

1. **Harvest and Resuspend the cells**

Harvest bacteria in capped 2.0ml collection tube by centrifugation at 10,000 X g (≥13,000 rpm) for 5 min at 4°C. (Do not use more than $1x10^7$ bacteria). Remove the culture medium and discard.

Resuspend the bacterial pellet in 100µl of lysozyme containing TE Buffer and mix well by gentle vortexing till no cell clumps are visible.
(Prepare lysozyme containing TE Buffer as indicated in General Preparation Instructions)

**Incubate the resuspended cells at 37°C for 30 minutes. Incubate on shaker or vortex 20 seconds for every 2 minutes during incubation**

2. **Lysis**
   Add 350µl or 600µl of RNA Lysis Solution (HRL) (DS0037) to the sample. Vortex or pipet to mix.

   **NOTE:** If insoluble material is visible, centrifuge for 2 minutes at 13,000rpm at room temperature (15-25°C), and use only the supernatant in the subsequent steps.

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

| Number of pelleted cells | RNA Lysis Solution (HRL) |
|---|---|
| $<5x10^6$ | 350µl |
| $5x10^6$ - $1x10^7$ | 600µl |

3. Pipet the lysate directly into a HiShredder (DSCA02) placed in a 2ml collection tube, and centrifuge at (≥13,000 rpm) for 2 minutes at room temperature (15-25°C). Collect the flow-through in the fresh collection tube (not provided) and proceed for binding step.

4. **Prepare for binding:**
   Add 280µl of ethanol (96-100%) to the cleared lysate, and mix immediately by pipetting. Do not centrifuge.

   **NOTE:** A precipitate may form after the addition of ethanol, but this will not affect the procedure.

5. **Load Lysate in HiElute Miniprep Spin Column (DBCA02)**
   Apply sample including any precipitate that may have formed, on the HiElute Miniprep Spin Column (Capped). Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) at room temperature (15-25°C). Discard the flow-through.

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

6. **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 HiElute Miniprep Spin Column as indicated above. Instead of continuing with the Pre Wash Solution (RW1) in step 7, follow steps I – IV below.

I. Pipet 350µl of Pre Wash Solution (RW1) (DS0041) into the HiElute Miniprep Spin column, and centrifuge for 1 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 6c.

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

III. Add 80µl of DNase I/ Digest Buffer mixture directly onto the HiElute Miniprep Spin Column. Incubate at room temperature (15-25°C) for 15 minutes.

IV. Pipet 350µl of Pre Wash Solution (RW1) into the HiElute Miniprep Spin column, and centrifuge for 1 minute at centrifuge 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.**

7. **Pre Wash**
   Add 700µl of Prewash Solution (RW1) to the HiElute Miniprep Spin Column centrifuge at ≥8000 x g (≥10,000 rpm) for 1 minute at room temperature (15-25°C). Discard the flow-through. Reuse the collection tube in step 8.

8. Transfer the HiElute Miniprep Spin column into a 2ml collection tube. Pipet 500µl of diluted Wash Solution (WS) (DS0012) **(Refer General Preparation Instructions)**. Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) at room temperature (15-25°C) to wash the column. Discard the flow-through.

9. Add another 500µl 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) at room temperature (15-25°C) to dry the membrane.

10. 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 ≥10000 x g (≥13,000 rpm) at room temperature (15-25°C) for 1 minute.

11. **RNA Elution**
    Transfer the HiElute Miniprep Spin column (capped) to a new uncapped 2ml collection tube. Pipet 30-50 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin 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 in to new 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.

**Precautions**

Read the procedure carefully before starting the experiment.

**Performance and Evaluation**

Each lot of HiMedia's HiPurA® Bacterial RNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

**Quality Control**

| Type of Sample | RNA Yield | RNA Purity |
|---|---|---|
| DH5α | Upto 40 µg | 1.8-2.1 |

**Trouble shooting Guide:**

| Sr. No. | Problem | Possible Cause | Solution |
|---|---|---|---|
| 1. | Clogged HiElute Miniprep Spin Column (Capped). | Too much starting material | In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material. |
| | | Centrifugation before adding ethanol not performed | Centrifuge the lysate before adding ethanol and use only this supernatant in subsequent steps. Pellets contain cell debris that can clog the HiElute Miniprep Spin Column (Capped). |
| | | Centrifugation temperature 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 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 HiElute Miniprep Spin Column | Repeat RNA elution, but incubate the HiElute Miniprep Spin Column for 10 mins 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 at 2 min at ≥8000 x g (≥10, 000 rpm) 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 full speed. |
| | | Incomplete removal of cell culture medium. | When processing cultured cells ensure complete removal of the cell culture medium after harvesting cells. |
| 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 Pre Wash Solution (RW1) | In subsequent preparations, incubate the HiElute Miniprep Spin Column (Capped) for 5 min at room temperature after the addition of Pre Wash Solution (RW1) and 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 HiElute Miniprep Spin Column (Capped) membrane by centrifugation at ≥8000 x g (≥10,000 rpm) for 2 minute to dry the membrane. Following the centrifugation, remove the HiElute Miniprep Spin 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

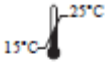
Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

## 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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