MB612 HiPurA™ 96 Total RNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB612		
		1X96 Preps	4X96 Preps	12X96 Preps
DS0037	RNA Lysis Solution (HRL)	65 ml	260 ml	2 x 390 ml
DS0041	Prewash Solution (RW1)	80 ml	320 ml	2 x 450 ml
DS0012	Wash Solution Concentrate (WS)	30 ml	120 ml	360 ml
DS0042	Elution Solution (RNase- Free Water)	20 ml	50 ml	140 ml
RBPL-96-01	HiPurA™ 96-well RNA Plate	1 no.	4 nos.	12 nos.
LWB-16-96	HiPurA™ 96-well Block (1.6 ml)	3 nos.	12 nos.	36 nos.
PR11	HiPurA™ Silicon Pad for sealing	1 no.	2 nos.	2 nos.
RBH-96-01	HiPurA™ 96-well HiShredder	1 no.	4 nos.	12 nos.
RVB-96	HiPurA™ 96-well V–Plate	1 no.	4 nos.	12 nos.

Introduction

HiPurA™ 96 Total RNA 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. HiMedia's 96-well format allows rapid processing of multiple samples. The RNA obtained is compatible with various downstream applications as mentioned above.

HiPurA™ 96 Total RNA Purification Kit

This kit simplifies isolation of RNA from animal cells, animal tissues, and for cleanup of RNA from crude enzymatic reactions (eg. DNase digestion, Proteinase digestion, RNA ligation and labeling reaction) by a simple procedure presented in 96-well format. The lysis buffer provided in the kit helps in cell disruption and denaturation; samples (in case of animal tissues) are passed through a HiPurA™ 96-well HiShredder which removes insoluble material and reduces the viscosity of the lysate by disrupting viscous material. Ethanol is added to the flow-through fraction, which promotes selective binding of RNA to the HiPurA™ 96-well RNA Plate. 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.

HiPurA™ 96-well RNA Plate (RBPL-96-01)

HiPurA™ 96-well RNA Plate is based on the advanced silica binding principle presented in a centrifugation and vacuum format. The system efficiently couples the reversible nucleic acid-binding properties of the advanced silica membrane to yield high quantity of RNA.

ISO 9001-2008 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2003 CERTIFIED

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It facilitates the binding, washing and elution steps thus enabling multiple samples to be processed simultaneously. It 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 during wash steps, leaving pure nucleic acids to be eluted in the buffer 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. The eluate 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 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™ 96 Total 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

- For processing of 96 different tissue samples, use of Tissue Lyser System for disruption and homogenization is recommended.
- Tabletop Centrifuge with 96-well rotor and plate carriers, capable of attaining at least 3,700 rpm or Vacuum Manifold for 96 well plate
- Vacuum source regulator (pump) (capable of giving negative pressure of -25 to -30 inches Hg)
- Vacuum regulator
- Multi-channel pipette
- 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 (procured from any standard company)

Storage

Store the HiPurA™ 96 Total RNA Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 1 year.

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 RNA Lysis Solution (HRL) (DS0037) 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 upto 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 nuclease-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 %)
1X96	30 ml	90 ml
4X96	120 ml	360 ml
12X96	360 ml	1080 ml

   NOTE: It is recommended for the user to a separate bottle (not provided) for Wash Solution Concentrate dilutions.

Centrifugation

All centrifugation steps are carried out in conventional laboratory centrifuge e.g. Beckman CS-6KR, Heraeus Varifuge 3.0R, or Sigma 6k10 with swinging bucket rotor with adaptors and carriers for 96-weel format. The 96-well accessories provided with the kit are compatible with almost all laboratory centrifuges and rotors. All centrifugation steps are performed at room temperature 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.

General Note:

1. If less than 96 samples are processed at a time, seal the unused wells properly with the provided HiPurA™ Silicon Pad for sealing (PR11).
2. The HiPurA™ Silicon Pad for sealing (PR11) used in the protocol should not be discarded. It can be reused after wiping with ethanol and washing properly.
3. Vacuum as well as centrifugation protocol can be performed for HiPurA™ 96 Total RNA Purification Kit. For a vacuum protocol, negative pressure of 30 mm Hg is required to achieve optimum results.

Procedure

NOTE: Generally, DNase digestion is not required since the solutions of this kit efficiently remove most of the DNA without DNase treatment. However, if further DNase treatment is required, before proceeding to wash steps, follow **DNase Digestion** mentioned in **Appendix.**

A. Protocol for Isolation of Total RNA from Animal Cells

1. Harvesting cells

a) Cells grown in a monolayer

Cells grown in a monolayer in multiwell cell-culture plates can be lysed directly in the wells. Completely remove medium by pipetting, and continue with step 2.

NOTE: Incomplete removal of the supernatant will dilute RNA Lysis Solution (HRL) in subsequent steps, inhibiting lysis and the binding of RNA to the HiPurA™ 96-well RNA Plate membrane. This will lead to reduced yield.

b) Cells grown in suspension

Transfer aliquots of up to 5 x 10^5 cells into the wells of a 96-well microplate. Centrifuge for 5 minutes at 300 x g (≈1,500 rpm) and completely remove supernatant by pipetting. Proceed with step 2.

NOTE: Incomplete removal of the supernatant will dilute RNA Lysis Solution (HRL) in subsequent steps, inhibiting lysis and the binding of RNA to the HiPurA™ 96-well RNA Plate membrane. This will lead to reduced yield.

2. Add 150 µl of RNA Lysis Solution (HRL) **(Refer General Preparation Instructions)** to each well of the microplate. Keeping the microplate flat on the bench, shake it vigorously back and forth for few seconds. While continuing to keep the plate flat on the bench, rotate the plate by 90° and shake it for few more seconds.

NOTE: After the addition of ethanol in step 3, total volume will be 300 µl. If the surface of the liquid is too high for convenient use in the 96-well microplate used, the volume of RNA Lysis Solution (HRL) may be reduced to 100 µl.

3. Add 1 volume (150 µl) of 70% ethanol and mix by pipetting. Proceed to binding, wash and elution steps using vacuum based protocol or centrifugation based protocol.

NOTE: Add 100 µl of 70% ethanol if 100 µl of RNA Lysis Solution (HRL) has been used in step 2.

B. Protocol for Isolation of Total RNA from Animal Tissues (Vacuum based)

Disruption and homogenization of tissue samples using Tissue Lyser System is recommended.

1. To each well of HiPurA™ 96-well Block (1.6 ml) (LWB-16-96) containing ground tissue samples (upto 30 mg), immediately add appropriate amount of RNA Lysis Solution (HRL) (DS0037) **(Refer General Preparation Instructions)** and mix thoroughly by pipetting. (Refer the table below for amount of RNA Lysis Solution (HRL) to be added).

Amount of starting material	RNA Lysis Solution (HRL)
<20 mg	350 µl
20 mg to 30 mg	600 µl

2. Apply the samples into the wells of a HiPurA™ 96-well HiShredder (RBH-96-01) and seal the plate with HiPurA™ Silicon Pad (PR11).
3. Connect the vacuum manifold to the vacuum source. Remove the manifold top and place a HiPurA™ 96-well Block (1.6 ml) (LWB-16-96) into the manifold base to collect the flow-through liquid. Place the HiPurA™ 96-well HiShredder (containing the samples) onto the manifold top.
4. Turn on the vacuum source and adjust it to achieve -30 inches Hg of pressure. Continue to draw vacuum through the plates until no liquid remains in any of the wells of HiPurA™ 96-well HiShredder. Turn off the vacuum source and release the vacuum from inside of the vacuum manifold using the vacuum regulator. Remove the HiPurA™ 96-well HiShredder from the manifold and temporarily set it aside on a piece of absorbent toweling (tissue paper stacks) or plastic wrap.
5. Transfer the flow-through fraction from the above step to HiPurA™ 96-well Block (1.6 ml) (LWB-16-96) without disturbing the cell debris pellet.
6. Add 1 volume of 70% ethanol and mix by pipetting. Proceed to binding, wash and elution steps using vacuum based protocol.

C. Protocol for Isolation of Total RNA from Animal Tissues (Centrifugation based)

Disruption and homogenization of tissue samples using Tissue Lyser System is recommended.

1. To each well of HiPurA™ 96-well Block (1.6 ml) (LWB-16-96) containing ground tissue samples (upto 30 mg), immediately add appropriate amount of RNA Lysis Solution (HRL) **(Refer General Preparation Instructions)** and mix thoroughly by pipetting (Refer the table below for amount of RNA Lysis Solution (HRL) to be added).

Amount of starting material	RNA Lysis Solution (HRL)
<20 mg	350 µl
20 mg to 30 mg	600 µl

2. Apply the samples into the wells of a HiPurA™ 96-well HiShredder (RBH-96-01) placed upon a new HiPurA™ 96-well Block (1.6 ml) (LWB-16- 96) and seal the HiShredder plate with HiPurA™ Silicon Pad (PR11). Centrifuge for 5 minutes at 2250 x g (3,700 rpm).
3. Transfer the flow-through fraction from step 6 to a new HiPurA™ 96-well Block (1.6 ml) (LWB-16- 96) without disturbing the cell debris pellet.
4. Add 1 volume of 70% ethanol and mix by pipetting. Proceed to binding, wash and elution steps using centrifugation based protocol.

D. Protocol for RNA Cleanup

1. Adjust volume of each sample to 100 µl with RNase-free water. Add 350 µl of RNA Lysis Solution (HRL) **(Refer General Preparation Instructions)** to each sample and mix by pipetting using a multichannel pipette.

2. Add 250 µl of ethanol (96–100%) to each sample and mix by pipetting. Proceed to binding, wash and elution steps using vacuum based protocol or centrifugation based protocol.

I) RNA Isolation from Animal Cells, Animal tissues and for RNA Cleanup- binding, washing and elution steps (Vacuum Based Protocol)

a. Apply the samples obtained after ethanol addition, to the HiPurA™ 96-well RNA Plate (RBPL-96-01) and place the HiPurA™ Silicon Pad (PR11) to seal the plate. Connect the vacuum manifold to the vacuum source. Remove the manifold top and place a HiPurA™ 96-well Block (1.6 ml) (LWB-16-96) into the manifold base to collect the flow-through liquid. Place the HiPurA™ 96-well RNA Plate containing the samples onto the manifold top.

b. Turn on the vacuum source and adjust it to -30 inches Hg. Continue to draw vacuum through the HiPurA™ 96-well RNA Plate until no liquid remains in any of the wells of the plate.

c. Turn off the vacuum source and release the vacuum from inside of the vacuum manifold using the vacuum regulator. Remove the HiPurA™ 96-well RNA Plate from the manifold and temporarily set it aside on a piece of absorbent towel (tissue paper stacks) or plastic wrap. Discard the flow-through and reuse the 1.6 ml block.

d. Reassemble the HiPurA™ 96-well RNA plate and 1.6 ml block in the vacuum manifold and add 700µl of Prewash Solution (RW1) (DS0041) to each well of the plate and place the silicon pad to seal the plate. Turn on the vacuum source and continue to draw vacuum through the plate until no liquid remains in any of the wells of the HiPurA™ 96-well RNA Plate.

e. Turn off the vacuum source and release the vacuum from inside of the vacuum manifold using the vacuum regulator. Remove the HiPurA™ 96-well RNA Plate from the manifold and temporarily set it aside on a piece of absorbent towel (tissue paper stacks) or plastic wrap. Discard the flow-through and reuse the 1.6 ml block.

f. Add 500µl of diluted Wash Solution (WS) **(Refer General Preparation Instructions)** to each well of the HiPurA™ 96-well RNA Plate placed on 1.6 ml block, place the silicon pad to seal the plate and turn on vacuum source. Apply vacuum until no liquid remains in any of the wells of the plate.

g. Turn off vacuum, and remove the HiPurA™ 96-well RNA Plate from the manifold and temporarily set it aside on a piece of absorbent towel (tissue paper stacks) or plastic wrap. Discard the flow-through and reuse the 1.6 ml block. Repeat this wash step one more time.

h. Discard the flow-through liquid and apply vacuum at a maximum of –30 inches Hg for 10 minutes to remove the traces of ethanol present in the Wash Solution.

i. Turn off the vacuum source. Remove the HiPurA™ 96-well RNA Plate from the vacuum manifold and vigorously tap the plate approximately 6-8 times on several layers of absorbent towel. Be careful not to damage the drip directors on the underside of the plate.

   NOTE: Lint-free absorbent towel is recommended to avoid the release of tiny fibres, which could contaminate the DNA and interfere with subsequent downstream applications.

j. Remove the HiPurA™ 96-well Block (1.6 ml) (LWB-16-96), which was used as a collection tray, from the vacuum manifold base and replace it with a new HiPurA™ 96-well V-Plate (RVB-96) for elution. Reassemble the manifold top and place the HiPurA™ 96-well RNA Plate on the HiPurA™ 96-well V-Plate. Add 50 µl of Elution Solution (RNase-Free Water) to the corresponding wells of HiPurA™ 96-well RNA Plate and incubate at room temperature (15-25°C) for 5 minutes. Turn on the vacuum source and allow the vacuum (-30 Hg pressure) to continue for 10 minutes.

k. Turn off the vacuum source and use the vacuum regulator to gradually release the vacuum from the manifold.

NOTE: Ensure that the Elution Solution (RNase-Free Water) is added directly onto the HiPurA™ 96-well RNA Plate.

l. Repeat the elution step with another 50 µl of Elution Solution (RNase-Free Water). Disassemble the manifold and remove the HiPurA™ 96-well V-Plate (RVB-96), which contains eluted RNA samples.

NOTE: Repeating the elution step is required for complete recovery of RNA.

NOTE: Elution with volumes less than 100 µl increases the final RNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. Storing RNA in water can cause acid hydrolysis.

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.

II) RNA Isolation from Animal Cells, Animal tissues and for RNA Cleanup- binding, washing and elution steps (Centrifugation Based Protocol)

a. Transfer the lysate obtained after ethanol addition, onto a HiPurA™ 96-well RNA Plate (RBPL-96-01) [placed on HiPurA™ 96-well Block (1.6 ml) (LWB-16-96)]. Place the HiPurA™ Silicon Pad (PR11) to seal the plate and centrifuge at 2250 x g (3,700 rpm) for 5 minutes. Discard the flow-through liquid and place the HiPurA™ 96-well RNA Plate (RBPL-96-01) in the same 1.6 ml block.

b. Add 700 µl of Prewash Solution (RW1) (DS0041) to the HiPurA™ 96-well RNA Plate, seal the plate with silicon pad and centrifuge at 3,700 rpm for 5 minutes. Discard the flow-through liquid and re-use the same 1.6 ml block.

c. Add 500 µl of diluted Wash Solution (WS) (DS0012) **(Refer General Preparation Instructions)** to the HiPurA™ 96-well RNA Plate, seal the plate with silicon pad and centrifuge for 5 minutes at 3,700 rpm. Discard the flow-through and place the HiPurA™ 96-well DNA Plate in the same 1.6 ml block. Repeat the wash step one more time.

d. Centrifuge the empty plate again at same speed for the additional 10 minutes to dry the plate to ensure complete removal of any residual wash solution.

e. Remove the HiPurA™ 96-well Block (1.6 ml) (LWB-16-96) which was used as collection tray and replace it with a new HiPurA™ 96-well V-Plate (RVB-96) for elution. Place the HiPurA™ 96-well RNA Plate on the HiPurA™ 96-well V-plate. Add 50 µl of Elution Solution (RNase-Free water) (DS0042) to the corresponding wells of HiPurA™ 96-well RNA Plate and incubate at room temperature (15-25°C) for 5 minutes. Centrifuge at 3,700 rpm for 5 minutes to elute the DNA. Repeat the elution step one more time.

NOTE: Repeating the elution step is required for complete recovery of RNA.

NOTE: Elution with volumes less than 100 µl increases the final RNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. Storing RNA in water can cause acid hydrolysis.

Appendix:

Optional: 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 HiPurA™ 96-well RNA Plate as mentioned in protocols. Instead of continuing with the Prewash Solution (RW1), follow steps below:

a. Pipet 350 µl of Prewash Solution (RW1) into the HiPurA™ 96-well RNA Plate placed on 1.6 ml block, seal the plate using silicon pad (provided) and centrifuge for 2 minutes at 2250 x g (3,700 rpm) or apply vacuum until no liquid remains in any of the wells of the plate. Discard the flow-through and reuse the 1.6 ml block in step c.

b. Add 1 ml of DNase I Solution to 7 ml of DNase Digest Buffer. Mix by gentle shaking or inversion. Do not vortex.

c. Add 80 µl of DNase I / Digest Buffer mixture directly onto the plate. Incubate at room temperature (15-25°C) for 15 minutes.

d. Pipet 350 µl of Prewash Solution (RW1) into the HiPurA™ 96-well RNA Plate, seal the plate using silicon pad and centrifuge for 2 minutes at 2250 x g (3,700 rpm) or apply vacuum until no liquid remains in any of the wells of the plate. Discard the flow-through and continue with wash, drying and elution steps as mentioned in protocols above.

Or

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

Precautions

Read the procedure carefully before starting the experiment.

Performance and Evaluation

Each lot of HiMedia's 96 Total RNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Purity
HeLa Cells	1.8-2.1

Trouble shooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Clogged HiPurA™ 96-well RNA Plate	Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see protocols).

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 (see protocols).
		Incomplete removal of supernatant	Ensure complete removal of the supernatant after harvesting cells (during RNA isolation from animal cells).
		Buffer temperatures too low	All buffers must be at room temperature (15-25°C) throughout the procedure.
		RNase contamination	Check for RNase contamination of buffers. Although all buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be careful not to introduce any RNase during the procedure or later handling.
		Elution volume too low	Repeat the elution step (see protocols).
3.	Low A_{260} / A_{280}	Water has been 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.	RNA degraded	Inappropriate handling of starting material	Ensure that samples are properly handled and that the protocol has been performed without interruptions, especially the initial steps involving lysis.
		RNase contamination	Check for RNase contamination of buffers. Although all buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be careful not to introduce any RNase during the procedure or later handling.
5.	RNA does not perform well in downstream experiments	Ethanol carryover	After the second Wash using Wash Solution (WS), be sure to dry the HiPurA™ 96-well RNA Plate membrane as mentioned in the procedure. Also, the RNA plate should not come in contact with the flow-through liquid.
		Salt carryover during elution	Ensure that Wash Solution (WS) is at room temperature (15-25°C). If using vacuum technology, be sure to tap the bottom side of the 96-well RNA Plate repeatedly on a stack of paper towels until no further liquid is released
		Elution volume too low	Use elution volumes as mentioned in the procedure. Repeat the elution step (see protocols).
		Vacuum pressure too low	A vacuum source capable of generating a vacuum pressure of -30 inches Hg is necessary to achieve efficient RNA binding to the membrane, washing, and elution when using vacuum or vacuum/spin technology.

6.	DNA contamination in downstream experiments	No incubation with Prewash Solution (RW1)	In subsequent preparations, incubate the HiPurA™ 96-well RNA Plate for 5 minutes at room temperature (15-25°C) after the addition of Prewash Solution (RW1) before centrifuging.
		No DNase treatment	Follow the optional DNase digestion step.

Safety Information

This 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 proper cleaning of equipment and floors with plenty of water. Offer surplus and non-recyclable solutions to a licenced disposal company.

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.

Consult instructions for use

Do not use if package is damaged

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Disclaimer :

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