MB619 HiPurA® Multi- Sample RNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB619	
		20 Preps	50 Preps
DS0044	RNA- XPress™ Reagent	24 ml	60 ml
DS0078	Binding Solution (PBR)	24 ml	60 ml
DS0037	RNA Lysis Solution (HRL)	24 ml	60 ml
DS0041	Prewash Solution (RW1)	20 ml	50 ml
R075	10X RBC Lysis Buffer Solution	24 ml	60 ml
DS2281	Lysozyme	80 mg	200 mg
DS0012	Wash Solution Concentrate (WS)	8 ml	20 ml
DS0192	Carrier RNA	0.28 mg	0.7 mg
DS0042	Elution Solution (RNase- Free Water)	3 ml	7.5 ml
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20 nos	50 nos
DSCA02	HiShredder [in DBCA016 Collection Tube]	20 nos	50 nos
DBCA025	Micro Centrifuge Tube-B (1.5 ml)	20 nos	50 nos
DBCA016	Collection Tube (Uncapped), Polypropylene (2.0 ml)	20 nos	50 nos
DBCA017	Collection Tube, Polypropylene (2.0 ml)	40 nos	100 nos

Intended Use

Recommended for isolation of RNA from human blood/animal blood, plasma, serum bacterial culture, yeast culture, Fungi, Plant, Virus and other samples such as cells, tissues, stool sample and body fluids.

Introduction

HiPurA® Multi- Sample 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. 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.

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

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HiPurA® Multi- Sample RNA Purification Kit

This kit simplifies isolation of RNA from human blood/animal blood, plasma, serum bacterial culture, yeast culture, Fungi, plant, virus and other samples such as cells, tissues, stool sample and body fluids and for cleanup of RNA from crude enzymatic reactions (e.g. DNase digestion, Proteinase digestion, RNA ligation, and labeling reaction) with spin-column procedure. The lysis buffer provided in the kit helps in c e l l disruption and denaturation, samples are centrifuged through 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.
A specialized high-salt buffer system allows upto 100g of total RNA longer than 200 bases to bind to the HiElute Miniprep Spin column (Capped) membrane.

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

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™ Multi- Sample 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) at 4°C
- RNase – free pipette tips (aerosol barrier recommended)
- Ethanol (96 – 100%)
- Ethanol (70%)
- Mortar and pestle
- Liquid nitrogen
- Chloroform (Product Code: MB109)
- Nuclease-free 15 ml centrifuge tubes
- 2- mercaptoethanol (ß -ME) (Product Code: MB041)
- Deoxyribonuclease I Solution (RNase-Free) (ML068)
- Molecular Biology Grade Water (ML024)
- 30°C water bath
- 37°C water bath

Storage

HiPurA™ Multi- Sample 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 amount is 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 240ºC 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 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 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	Ethanol (96-100 %)
20	8 ml	24 ml
50	20 ml	60 ml

5. **Dilute 10X RBC Lysis Buffer Solution (R075) as follows:**

Number of Preps	10X RBC Lysis Buffer Solution	RNase- Free Water
20	24 ml	216 ml
50	60 ml	540 ml

6. **For Bacterial protocol**

   Prepare Lysozyme Solution (Product Code: DS2281)
   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.

7. **For Plant & Fungal protocol**

   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.

8. For Virus protocol

I. Preparation of Carrier RNA

Number of Preps	Carrier RNA	Elution Buffer (RNase free water)
20	0.28 mg	280 µl
50	0.7 mg	700 µl

Dissolve Carrier RNA thoroughly by pipetting. We recommend storing the reconstituted Carrier RNA at -20°C in aliquots to avoid repeated freeze and thaw.

II. Preparation of Carrier RNA –Lysis Solution (HRL)

Number of Preps	Volume of Carrier RNA	Volume of Lysis Solution (HRL)
20	112 µl	11.2 ml
50	280 µl	28 ml

NOTE: Concentration of Carrier RNA to be used is 10µg/ml

Calculate the volume of Carrier RNA –Lysis Solution (HRL) as follows:

a X 0.56 ml = **b** ml

b ml X 10 µl/ml = **c** µl

Where, **a** = number of samples to be processed

b = volume of Lysis Solution (HRL) to be added for '**a**' number of samples

c = volume of Carrier RNA to be added to Lysis Buffer (HRL)

eg: for 2 number of samples, add 1.12 ml of Lysis Solution (HRL) and 11.2 µl of Carrier RNA

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.

Specimen Handling and Collection

Collect whole blood in an anticoagulant tube (an EDTA tube is preferred) under sterile conditions (if to be used for future) and store the samples at 2-8°C for short term storage or - 20°C for long term storage.

Collect cells, tissues, Serum, Plasma, stool sample and body fluids in a clean sterile container and store the samples at 2-8°C for short term storage or -20°C for long term storage. Repeated freeze- thaw of samples should be avoided.

Ensure that the blood/ tissue/ cells/stool/bacterial culture/yeast Culture, Serum, Plasma and body fluids sample is at room temperature (15- 25°C) before beginning the protocol. After use, contaminated material must be sterilized by autoclaving before discarding.

Types of Specimen

Clinical samples: Whole blood, tissues, Bacterial culture, Serum, Plasma, yeast culture, Fungi,

Plant, Virus, stool and body fluids.

Procedure

A. <u>Protocol for Isolation of RNA from Blood</u>

Specimen Handling and Collection
IMPORTANT
Equilibrate the 1X RBC Lysis Buffer Solution to Room Temperature (15-25°C) before starting.

1. Preparation of sample (For Erythrocyte Lysis)
   (Prepare 1X RBC Lysis Buffer Solution as indicated in General Preparation Instructions)
   Mix 1 volume of whole blood with 5 volumes of 1X RBC Lysis Buffer Solution (R075) in Micro Centrifuge Tube-B (1.5 ml) (provided). For example, to 200 µl of whole blood, add 1 ml of 1X RBC Lysis Buffer Solution.

   For optimal results the volume of mixture (Blood + 1X RBC Lysis Buffer Solution) should not exceed ¾ of the volume of the tube to allow efficient mixing. For example, add 5 ml of 1X RBC Lysis Buffer Solution to 1 ml of whole blood, and mix in a tube which has the total volume of ≥8 ml. Whole Blood treated with any common anticoagulant such as heparin or EDTA can be used in this protocol.

   NOTE: Use an appropriate amount of whole blood. Upto 1.5 ml of healthy blood (typically 4000-7000 leukocytes per microliter) can be processed. Reduce amount appropriately if blood with elevated numbers of leukocytes is used. (In this case, also adjust amount of RNA Lysis Solution (HRL) in step 6).

   A common alternative to erythrocyte lysis is HiSep™ LSM 1077, Lymphocyte Separation Media (LS001). In contrast to erythrocyte lysis procedures, HiSep™ LSM 1077 offers a quick and reliable method for the simple isolation of human mononuclear cells and lymphocytes. Mononuclear cells isolated using HiSep™ LSM can be processed with Blood RNA Purification Kit.

2. Incubate for 10-15 minutes on ice. Mix by vortexing briefly 2 times during incubation. The cloudy suspension becomes translucent during incubation, indicating lysis of erythrocytes. If necessary, incubation time can be extended to 20 minutes.

3. Centrifuge at 400 x g (1,700 rpm) for 10 minutes at 4°C, and completely remove and discard supernatant.

   NOTE: Leukocytes will form a pellet after centrifugation. Ensure supernatant is completely removed.

4. Repeat lysis step with the cell pellet, by adding 2 volumes of 1X RBC Lysis Buffer Solution per 1 volume of whole blood used in step1. For example, to 200 µl of whole blood, add 400 µl of 1X RBC Lysis Buffer Solution. Thoroughly vortex to resuspend the cells.

5. Centrifuge at 400 x g (1700 rpm) for 10 minutes at 4°C, and completely remove and discard supernatant. Incomplete removal of the supernatant will interfere with lysis and subsequent binding of RNA to the HiElute Miniprep Spin Column, resulting in lower yield.

6. **Lysis reaction**
   Add RNA Lysis Solution (HRL) (DS0037) **(Refer General Preparation Instructions)** to pelleted leukocytes according to the table below. Vortex or pipet to mix.

When not using healthy blood, refer to number of leukocytes to determine the volume of RNA Lysis Solution (HRL) required. RNA Lysis Solution (HRL) disrupts the cells. No cell clumps should be visible before you proceed to the homogenization step. Vortex or pipet further to remove any clumps.

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

RNA Lysis Solution (HRL)	Whole blood (ml)	Number of leukocytes
350 µl	Upto 0.5	Upto 2 $X10^6$
600 µl	0.5 to 1.5	2 $X10^6$ to 1 $X\ 10^7$

7. Pipet the lysate directly into a HiShredder (DSCA01) placed in a 2 ml collection tube, and centrifuge for 2 min at full speed to homogenize.

   NOTE: If too many cells have been used, after homogenization the lysate will be too viscous to pipet. In this case divide the sample into two aliquots and adjust the volume of each aliquot to 600 µl with RNA Lysis Solution (HRL). Continue with Binding step.

8. Pipet the lysate directly into a HiShredder (DSCA01) placed in a 2 ml Collection tube, and centrifuge for 2 min at full speed to homogenize.

   NOTE: If too many cells have been used, after homogenization the lysate will be too viscous to pipet. In this case divide the sample into two aliquots and adjust the volume of each aliquot to 600 µl with RNA Lysis Solution (HRL).

9. **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 addition of ethanol. This does not affect the procedure.

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

    NOTE: If 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.

    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.

ii. Carryout lysis, homogenization, and loading onto the HiElute Miniprep Spin Column (Capped) as indicated above. Instead of continuing with the Prewash Solution (RW1), follow steps below.

iii. Pipet 350 µl of Prewash Solution (RW1) (DS0041) into the HiElute Miniprep Spin column (Capped), and centrifuge for 15 sec at 8000 x g (≈10,000 rpm). Discard the flow through and reuse the collection tube.

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

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

vi. Pipet 350 µl of Prewash Solution (RW1) into the HiElute Miniprep Spin column, and centrifuge for 15 seconds at centrifuge at 8000 x g (≈10,000 rpm). Discard the flow-through and continue with the Wash step.

Or

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

11. **Prewash Solution (RW1)**
    Add 700 µl of Prewash Solution (RW1) to the HiElute Miniprep Spin Column (Capped) centrifuge at ≥8000 x g (≥10,000 rpm) for 1 minute. Discard the flow-through. Reuse the collection tube.

12. **Wash**
    Pipet 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 W ash Solution Concentrate (WS).

13. Add another 500 µl of diluted Wash Solution (WS) to the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 2 minutes at ≥8000 x g (≥10,000 rpm) to dry the membrane. Continue directly with elution step or to eliminate any chance of possible Wash Solution carryover, perform the next step.

    Optional: Place the column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge at 13,000 X g (14,000 rpm) for 1 minute.

14. **RNA Elution**
    Transfer the HiElute Miniprep Spin column (Capped) to a new 2 ml capped collection tube. Pipet 30- 50 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) to elute.

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

16. Transfer the eluate to a fresh capped 2ml collection tube for longer RNA 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.

B. Protocol for Isolation of RNA from 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 1 X107 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)
$<5 \times 10^6$	350µl
$5X\ 10^6$- $1\ X10^7$	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 in Page No.13

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 (Capped) [DBCA03]**
Apply sample including any precipitate that may have formed, on the HiElute Miniprep Spin Column (Capped). Close the tube gently, and centrifuge for 15 seconds at 8000 x g (≈10,000 rpm). Discard the flow-through.

NOTE: If 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.

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.

i. Carryout lysis, homogenization, and loading onto the HiElute Miniprep Spin Column (Capped) as indicated above. Instead of continuing with the Prewash Solution (RW1), follow steps below.

ii. Pipet 350 µl of Prewash Solution (RW1) (DS0041) into the HiElute Miniprep Spin column (Capped), and centrifuge for 15 sec at 8000 x g (≈10,000 rpm). Discard the flow through and reuse the collection tube.

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

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

v. Pipet 350 µl of Prewash Solution (RW1) into the HiElute Miniprep Spin column, and centrifuge for 15 seconds at centrifuge at 8000 x g (≈10,000 rpm). Discard the flow-through and continue with the Wash step.

Or

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

6. **Prewash Solution (RW1)**
   Add 700 µl of Prewash Solution (RW1) to the HiElute Miniprep Spin Column (Capped) centrifuge at ≥8000 x g (≥10,000 rpm) for 1 minute. Discard the flow-through. Reuse the collection tube.

7. **Wash**
   Pipet 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 W ash Solution Concentrate (WS).

8. Add another 500 µl of diluted Wash Solution (WS) to the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 2 minutes at ≥8000 x g (≥10,000 rpm) to dry the membrane. Continue directly with elution step or to eliminate any chance of possible Wash Solution carryover, perform the next step.

   Optional: Place the column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge at 13,000 X g (14,000 rpm) for 1 minute.

9. **RNA Elution**
   Transfer the HiElute Miniprep Spin column (Capped) to a new 2 ml capped collection tube. Pipet 30- 50 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) to elute.

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

11. Transfer the eluate to a fresh capped 2ml collection tube for longer RNA 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.

C. Protocol for Isolation of RNA from Yeast

1. Grow yeast culture *Saccharomyces cerevisiae* or *Candida spp.* in YPD medium (Product Code: M1363). Harvest cells, maximum up to 1 $X10^8$ 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. **Resuspendcells**

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. 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.

3. **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. Pipet the lysate directly into a HiShredder (DSCA01) placed in a 2ml collection tube, and centrifuge for 2 minutes at maximum speed. Repeat the step no 5 for remaining lysate. Continue with Binding step.

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

5. **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 addition of ethanol. This does not affect the procedure.

6. **Load Lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Apply sample including any precipitate that may have formed, on the HiElute Miniprep Spin Column (Capped). Close the tube gently, and centrifuge for 15 seconds at 8000 x g (≈10,000 rpm). Discard the flow-through.

   NOTE: If 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.

   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.

i. Carryout lysis, homogenization, and loading onto the HiElute Miniprep Spin Column (Capped) as indicated above. Instead of continuing with the Prewash Solution (RW1), follow steps below.

ii. Pipet 350 µl of Prewash Solution (RW1) (DS0041) into the HiElute Miniprep Spin column (Capped), and centrifuge for 15 sec at 8000 x g (≈10,000 rpm). Discard the flow through and reuse the collection tube.

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

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

v. Pipet 350 µl of Prewash Solution (RW1) into the HiElute Miniprep Spin column, and

centrifuge for 15 seconds at centrifuge at 8000 x g (≈10,000 rpm). Discard the flow-through and continue with the Wash step.

Or

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

7. **Prewash Solution (RW1)**
   Add 700 µl of Prewash Solution (RW1) to the HiElute Miniprep Spin Column (Capped) centrifuge at ≥8000 x g (≥10,000 rpm) for 1 minute. Discard the flow-through. Reuse the collection tube.

8. **Wash**
   Pipet 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 W ash Solution Concentrate (WS).

9. Add another 500 µl of diluted Wash Solution (WS) to the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 2 minutes at ≥8000 x g (≥10,000 rpm) to dry the membrane. Continue directly with elution step or to eliminate any chance of possible Wash Solution carryover, perform the next step.

   Optional: Place the column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge at 13,000 X g (14,000 rpm) for 1 minute.

10. **RNA Elution**
    Transfer the HiElute Miniprep Spin column (Capped) to a new 2 ml capped collection tube. Pipet 30- 50 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) to elute.

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

12. Transfer the eluate to a fresh capped 2ml collection tube for longer RNA 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.

D. Protocol for Isolation of RNA from Cells

1. **Harvest the cells according to step a, b, or c**

a. **Cells grown in suspension (do not use more than 1 x 10^7 cells):**
   Pellet up to 1 x 10^7 cells by centrifuging for 5 minutes at 300 x g (≈1500 rpm) in a Collection tube (not supplied). Discard the culture medium completely and continue with step 2 of Lysis reaction (Protocol for Isolation of RNA from Cells).

   NOTE: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the binding of RNA to the HiElute Miniprep Spin Column (Capped) membrane resulting in lower RNA yield.

b. **Cells grown in a monolayer (do not use more than 1 X 10^7 cells):**
Direct lysis of the cells can be done in the cell-culture vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. Cells grown in cell-culture flasks should always be trypsinized. Proceed immediately to step 2 of Lysis reaction (Protocol for Isolation of RNA from Cells).

c. **To lyse cells directly in culture dish:**
Determine the number of cells. Completely aspirate the cell-culture medium, and proceed immediately to step 2 of Lysis reaction (Protocol for Isolation of RNA from Cells).

NOTE: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the binding of RNA to the HiElute Miniprep Spin Column (Capped) membrane resulting in lower RNA yield.

To trypsinize and collect cells:
Determine the number of cells. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, and add 0.1–0.25% trypsin in PBS. After the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 300 x g for 5 minutes. Completely aspirate the supernatant, and proceed to step 2 of Lysis reaction.

NOTE: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the binding of RNA to the HiElute Miniprep Spin Column (Capped) membrane resulting in lower RNA yield

2. **Lysis reaction**

a. For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add the appropriate volume of RNA Lysis Solution (HRL) (DS0037) (see the table below). Vortex or pipet to mix, and continue with step 3.

NOTE: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields. Ensure that ß -ME is added to RNA Lysis Solution (HRL) before use.

Number of pelleted cells	RNA Lysis Solution (HRL)
<5X10^6	350 µl
5 X10^6- 1 X10^7	600 µl

b. For Cells grown in a monolayer, add appropriate volume of RNA Lysis Solution (HRL) (see the table below) to the cell culture dish. Collect cell lysate into a collection tube (not supplied). Vortex or pipet to mix and ensure that no cell clumps are visible before proceeding to step 3.

NOTE: Regardless of the cell number, use the Lysis Solution volumes indicated to completely cover the surface of the dish. **Ensure that ß -ME is added to RNA Lysis Solution (HRL) before use.**

Dish diameter	RNA Lysis Solution (HRL)
< 6 cm	350 µl
6-10 cm	600 µl

NOTE: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields.

3. Pipet the lysate directly into a HiShredder (DSCA02) placed in a 2 ml uncapped collection tube, and centrifuge for 2 minutes at 13,000 X g (14,000 rpm).

4. **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 addition of ethanol. This does not affect the procedure.

5. **Load Lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Apply sample including any precipitate that may have formed, on the HiElute Miniprep Spin Column (Capped). Close the tube gently, and centrifuge for 15 seconds at 8000 x g (≈10,000 rpm). Discard the flow-through.

   NOTE: If 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.

   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.

i. Carryout lysis, homogenization, and loading onto the HiElute Miniprep Spin Column (Capped) as indicated above. Instead of continuing with the Prewash Solution (RW1), follow steps below.

ii. Pipet 350 µl of Prewash Solution (RW1) (DS0041) into the HiElute Miniprep Spin column (Capped), and centrifuge for 15 sec at 8000 x g (≈10,000 rpm). Discard the flow through and reuse the collection tube.

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

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

v. Pipet 350 µl of Prewash Solution (RW1) into the HiElute Miniprep Spin column, and centrifuge for 15 seconds at centrifuge at 8000 x g (≈10,000 rpm). Discard the flow-through and continue with the Wash step.

Or

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

6. **Prewash Solution (RW1)**
   Add 700 µl of Prewash Solution (RW1) to the HiElute Miniprep Spin Column (Capped) centrifuge at ≥8000 x g (≥10,000 rpm) for 1 minute. Discard the flow-through. Reuse the collection tube.

7. **Wash**
   Pipet 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 W ash Solution Concentrate (WS).

8. Add another 500 µl of diluted Wash Solution (WS) to the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 2 minutes at ≥8000 x g (≥10,000 rpm) to dry the membrane. Continue directly with elution step or to eliminate any chance of possible Wash Solution carryover, perform the next step.

   Optional: Place the column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge at 13,000 X g (14,000 rpm) for 1 minute.

9. **RNA Elution**
   Transfer the HiElute Miniprep Spin column (Capped) to a new 2 ml capped collection tube. Pipet 30- 50 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) to elute.

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

11. Transfer the eluate to a fresh capped 2ml collection tube for longer RNA 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.

E. <u>Protocol for Isolation of RNA from Tissues</u>

1. **Fresh or frozen (up to 30 mg) tissue**.
   Immediately place the weighed (fresh or frozen) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Allow the liquid nitrogen to evaporate. Do not allow the samples to thaw.

2. **Lysis reaction**
   Add appropriate volume of RNA Lysis Solution (HRL) (DS0037) (see the table below). Mix properly and transfer it to an RNase free 2.0 ml capped collection tube. Vortex or pipet to mix.

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

NOTE: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields. **Ensure that ß -ME is added to RNA Lysis Solution (HRL) before use.**

3. Pipet the lysate directly into a HiShredder (DSCA02) placed in a 2 ml u ncapped collection tube, and centrifuge for 2 min at full speed.

4. **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 addition of ethanol. This does not affect the procedure.

5. **Load Lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Apply sample including any precipitate that may have formed, on the HiElute Miniprep Spin Column (Capped). Close the tube gently, and centrifuge for 15 seconds at 8000 x g (≈10,000 rpm). Discard the flow-through.

   NOTE: If 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.

   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.

vi. Carryout lysis, homogenization, and loading onto the HiElute Miniprep Spin Column (Capped) as indicated above. Instead of continuing with the Prewash Solution (RW1), follow steps below.

vii. Pipet 350 µl of Prewash Solution (RW1) (DS0041) into the HiElute Miniprep Spin column (Capped), and centrifuge for 15 sec at 8000 x g (≈10,000 rpm). Discard the flow through and reuse the collection tube.

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

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

x. Pipet 350 µl of Prewash Solution (RW1) into the HiElute Miniprep Spin column, and centrifuge for 15 seconds at centrifuge at 8000 x g (≈10,000 rpm). Discard the flow-through and continue with the Wash step.

Or

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

6. **Prewash Solution (RW1)**
   Add 700 µl of Prewash Solution (RW1) to the HiElute Miniprep Spin Column (Capped) centrifuge at ≥8000 x g (≥10,000 rpm) for 1 minute. Discard the flow-through. Reuse the collection tube.
7. **Wash**
   Pipet 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 W ash Solution Concentrate (WS).

8. Add another 500 µl of diluted Wash Solution (WS) to the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 2 minutes at ≥8000 x g (≥10,000 rpm) to dry the membrane. Continue directly with elution step or to eliminate any chance of possible Wash Solution carryover, perform the next step.

Optional: Place the column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge at 13,000 X g (14,000 rpm) for 1 minute.

9. **RNA Elution**
   Transfer the HiElute Miniprep Spin column (Capped) to a new 2 ml capped collection tube. Pipet 30- 50 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) to elute.

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

11. Transfer the eluate to a fresh capped 2ml collection tube for longer RNA 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.

F. <u>Protocol for Isolation of RNA from Virus</u>

1. Add 140 µl of cell free sample like serum, plasma or cell-free fluid to Collection Tube, Polypropylene (2.0 ml).

2. Add 560 µl of Carrier RNA-Lysis Solution (HRL) to the cell free sample. (Refer to General Preparation Instructions). Mix by pulse vortexing for 15 seconds.

3. Incubate for 10 minutes at room temperature (15-25°C). Centrifuge the samples for 10 seconds to remove droplets formed inside the cap of collection tubes.

4. Add 560 µl of ethanol (96-100%) to the sample, mix well by gentle aspiration.

5. **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 addition of ethanol. This does not affect the procedure.

6. **Load Lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Apply sample including any precipitate that may have formed, on the HiElute Miniprep Spin Column (Capped). Close the tube gently, and centrifuge for 15 seconds at 8000 x g (≈10,000 rpm). Discard the flow-through.

   NOTE: If 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.

   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.

xi. Carryout lysis, homogenization, and loading onto the HiElute Miniprep Spin Column (Capped) as indicated above. Instead of continuing with the Prewash Solution (RW1), follow steps below.

xii. Pipet 350 µl of Prewash Solution (RW1) (DS0041) into the HiElute Miniprep Spin column (Capped), and centrifuge for 15 sec at 8000 x g (≈10,000 rpm). Discard the flow through and reuse the collection tube.

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

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

xv. Pipet 350 µl of Prewash Solution (RW1) into the HiElute Miniprep Spin column, and centrifuge for 15 seconds at centrifuge at 8000 x g (≈10,000 rpm). Discard the flow-through and continue with the Wash step.

Or

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

7. **Prewash Solution (RW1)**
   Add 700 µl of Prewash Solution (RW1) to the HiElute Miniprep Spin Column (Capped) centrifuge at ≥8000 x g (≥10,000 rpm) for 1 minute. Discard the flow-through. Reuse the collection tube.

8. **Wash**
   Pipet 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 W ash Solution Concentrate (WS).

9. Add another 500 µl of diluted Wash Solution (WS) to the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 2 minutes at ≥8000 x g (≥10,000 rpm) to dry the membrane. Continue directly with elution step or to eliminate any chance of possible Wash Solution carryover, perform the next step.

   Optional: Place the column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge at 13,000 X g (14,000 rpm) for 1 minute.

10. **RNA Elution**
    Transfer the HiElute Miniprep Spin column (Capped) to a new 2 ml capped collection tube. Pipet 30- 50 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) to elute.

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

12. Transfer the eluate to a fresh capped 2ml collection tube for longer RNA 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.

G. Protocol for Isolation of RNA from Plant & fungus

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.

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).Add 0.5 volumes (usually 775 µl) of ethanol (96-100%) to the above solution.

5. **Load Lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Apply sample including any precipitate that may have formed, on the HiElute Miniprep Spin Column (Capped). Close the tube gently, and centrifuge for 15 seconds at 8000 x g (≈10,000 rpm). Discard the flow-through.

   NOTE: If 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.

   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.

i. Carryout lysis, homogenization, and loading onto the HiElute Miniprep Spin Column (Capped) as indicated above. Instead of continuing with the Prewash Solution (RW1), follow steps below.

ii. Pipet 350 µl of Prewash Solution (RW1) (DS0041) into the HiElute Miniprep Spin column (Capped), and centrifuge for 15 sec at 8000 x g (≈10,000 rpm). Discard the flow through and reuse the collection tube.

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

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

v. Pipet 350 µl of Prewash Solution (RW1) into the HiElute Miniprep Spin column, and centrifuge for 15 seconds at centrifuge at 8000 x g (≈10,000 rpm). Discard the flow-through and continue with the Wash step.

Or

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

6. **Prewash Solution (RW1)**
   Add 700 µl of Prewash Solution (RW1) to the HiElute Miniprep Spin Column (Capped) centrifuge at ≥8000 x g (≥10,000 rpm) for 1 minute. Discard the flow-through. Reuse the collection tube.

7. **Wash**
   Pipet 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 W ash Solution Concentrate (WS).

8. Add another 500 µl of diluted Wash Solution (WS) to the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 2 minutes at ≥8000 x g (≥10,000 rpm) to dry the membrane. Continue directly with elution step or to eliminate any chance of possible Wash Solution carryover, perform the next step.

   Optional: Place the column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge at 13,000 X g (14,000 rpm) for 1 minute.

9. **RNA Elution**
   Transfer the HiElute Miniprep Spin column (Capped) to a new 2 ml capped collection tube. Pipet 30- 50 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) to elute.

10. If the expected RNA yield is > 30 µg, repeat the elution step as described with a second

volume of RNase-Free Water. Elute into the same collection tube.

11. Transfer the eluate to a fresh capped 2ml collection tube for longer RNA 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.

H. Protocol for Isolation of Total RNA from Stool sample and Body fluids

1. Add 100mg stool sample or 100µl of body fluid to a clean collection tube, polypropylene (2.0ml).

2. **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. Ensure that ß -ME is added to RNA Lysis Solution (HRL) before use **(Refer General Preparation Instructions).**

3. Pipet the lysate directly into a HiShredder (DSCA02) placed in a 2 ml uncapped collection tube, and centrifuge for 2 minutes at 13,000 X g (14,000 rpm).

4. **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 addition of ethanol. This does not affect the procedure.

5. **Load Lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Apply sample including any precipitate that may have formed, on the HiElute Miniprep Spin Column (Capped). Close the tube gently, and centrifuge for 15 seconds at 8000 x g (≈10,000 rpm). Discard the flow-through.

   NOTE: If 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.

   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.

6. Carryout lysis, homogenization, and loading onto the HiElute Miniprep Spin Column (Capped) as indicated above. Instead of continuing with the Prewash Solution (RW1), follow steps below.

i. Pipet 350 µl of Prewash Solution (RW1) (DS0041) into the HiElute Miniprep Spin column (Capped), and centrifuge for 15 sec at 8000 x g (≈10,000 rpm). Discard the flow through and reuse the collection tube.

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

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

iv. Pipet 350 µl of Prewash Solution (RW1) into the HiElute Miniprep Spin column, and centrifuge for 15 seconds at centrifuge at 8000 x g (≈10,000 rpm). Discard the flow-through and continue with the Wash step.

Or

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

7. **Prewash Solution (RW1)**
   Add 700 µl of Prewash Solution (RW1) to the HiElute Miniprep Spin Column (Capped) centrifuge at ≥8000 x g (≥10,000 rpm) for 1 minute. Discard the flow-through. Reuse the collection tube.

8. **Wash**
   Pipet 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 W ash Solution Concentrate (WS).

9. Add another 500 µl of diluted Wash Solution (WS) to the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 2 minutes at ≥8000 x g (≥10,000 rpm) to dry the membrane. Continue directly with elution step or to eliminate any chance of possible Wash Solution carryover, perform the next step.

   Optional: Place the column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge at 13,000 X g (14,000 rpm) for 1 minute.

10. **RNA Elution**
    Transfer the HiElute Miniprep Spin column (Capped) to a new 2 ml capped collection tube. Pipet 30- 50 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) to elute.

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

12. Transfer the eluate to a fresh capped 2ml collection tube for longer RNA 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.

I. Protocol for RNA Cleanup

1. A maximum of 100 µg RNA can be used in the RNA cleanup protocol. Adjust sample to a volume of 100 µl with RNase-free water. Add 350 µl of RNA Lysis Solution (HRL) (DS0037) and mix thoroughly.

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

2. **Prepare for binding:**
   Add 250 µl of ethanol (96-100%) to the diluted RNA, and mix thoroughly by pipetting. Do

not centrifuge. Continue without delay with column step (below).

3. **Load Lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Apply sample including any precipitate that may have formed, on the HiElute Miniprep Spin Column (Capped). Close the tube gently, and centrifuge for 15 seconds at 8000 x g (≈10,000 rpm). Discard the flow-through.

   NOTE: If 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.

4. **Wash**
   Pipet 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 W ash Solution Concentrate (WS).

5. Add another 500 µl of diluted Wash Solution (WS) to the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 2 minutes at ≥8000 x g (≥10,000 rpm) to dry the membrane. Continue directly with elution step or to eliminate any chance of possible Wash Solution carryover, perform the next step.

   Optional: Place the column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge at 13,000 X g (14,000 rpm) for 1 minute.

6. **RNA Elution**
   Transfer the HiElute Miniprep Spin column (Capped) to a new 2 ml capped collection tube. Pipet 30- 50 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) to elute.

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

14. Transfer the eluate to a fresh capped 2ml collection tube for longer RNA 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)

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 clinical laboratory practices while handling clinical 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

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

Quality Control

Type of Sample	RNA Purity
Body Fluids/ Cells/ Tissue/ Virus/ Fungus/ Bacteria/ Yeast/ Plant	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 (see protocols).
		Tissues: Centrifugation before adding ethanol not performed	Centrifuge the lysate before adding ethanol and use only this supernatant in subsequent steps. Pellets contain cell debris can clog the HiElute Miniprep Spin Column (Capped).
		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 column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol containing lysate to 37°C before transferring it to the 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 (see protocols).
		RNA still bound to HiElute Miniprep Spin Column	Repeat RNA elution, but incubate the column for 10 minutes at room temperature with Elution solution (RNase free water) before centrifuging.

		Ethanol carryover	During the second wash with Wash Solution (WS) be sure to centrifuge at ≥8000 x g (≥10, 000 rpm) for 2 minutes 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
		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 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.	DNA contamination in downstream experiments	No incubation with Prewash Solution (RW1)	In subsequent preparations, incubate the HiElute Miniprep Spin Column (Capped) for 5 minutes at room temperature 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 HiElute Miniprep Spin Column membrane by centrifugation at ≥8000 x g (≥10,000 rpm) for 2 minutes to dry the membrane. Following the

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.

Symbols

	Manufacturer		Do not use if package is damaged
	Catalogue number		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Contains biological material of animal origin
	Batch code		

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