OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Total RNA Kit II

R6934-00	5 preps
R6934-01	50 preps
R6934-02	200 preps

August 2011

For research use only.Not intended for diagnostic testing.

E.Z.N.A.® Total RNA Kit II

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Manual Revision: August 2011

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Introduction

The E.Z.N.A.® Total RNA Kit II is designed for isolating total cellular RNA from tissues rich in fat such as brain adipose tissues. However, this kit can also be used for the isolation of total RNA from other type of tissues including cultured eukaryotic cells, animal tissues, or bacteria.

RNA purified using the E.Z.N.A.® Total RNA method is ready for applications such as RT-PCR*, Northern blotting, poly A+ RNA (mRNA) purification, nuclease protection, and *in vitro* translation.

The E.Z.N.A.® Total RNA Kit II uses the reversible binding properties of HiBind® matrix, a new silica-based material. By combining the high lysis efficiency of RNA-Solv Reagent with OBI's innovative HiBind® technology, this kit can extract total cellular RNA from all types of animal or human tissues including fatty tissues such as brain and adipose tissue. A specifically formulated high salt buffer system allows more than 100 µg RNA molecules greater than 200 bases to bind to the matrix. Cells or tissues are first homogenized with RNA-Solv Reagent that inactivates RNases. After adding chloroform, the homogenate is separated into aqueous and organic phase by centrifugation. The aqueous phase which contains the RNA is adjusted with ethanol and applied to the HiBind® RNA Mini Column to which total RNA binds, while cellular debris and other contaminants are washed away. High-quality RNA is eluted in DEPC Water.

For isolating total RNA below 200 nt use the miRNA isolation Kit (R7034). For isolating total RNA from gram-positive bacteria, the recommended kit is the Bacterial RNA Kit (R6950).

While this kit may be used for the isolation of RNA from whole blood, we recommend that you use the E.Z.N.A.® Blood RNA Kit (R6814) as it is specifically designed for effective hemolysis and hemoglobin removal, thereby giving higher RNA yields.

Binding Capacity

Each HiBind® RNA Mini Column can bind approximately 100 µg RNA. Using greater than 30 mg tissue or 1 x 10^7 cells is not recommended.

Centrifugation Protocols

Add RNA-Solv Reagent and Lyse

Homogenize

Add Chloroform

Transfer Aqueous Phase

Add Ethanol

Transfer to a HiBind® RNA Column

Wash 3x

Dry

Elute

Vacuum Protocol

Add RNA-Solv Reagent and Lyse

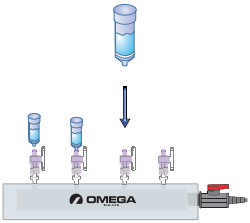
Homogenize

Add Chloroform

Transfer Aqueous Phase

Add Ethanol

Apply Sample to Column



Wash 3x

Dry

Elute

Kit Contents

E.Z.N.A.® Total RNA Kit II	5 Preps	50 Preps	200 Preps
Product Number	R6934-00	R6934-01	R6934-02
Purification	5	50	200
HiBind® RNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
RNA-Solv Reagent	5 x 1 mL	2 x 28 mL	9 x 25 mL
RNA Wash Buffer I	5 mL	50 mL	200 mL
RNA Wash Buffer II	5 mL	12 mL	50 mL
DEPC Water	1.0 mL	10 mL	40 mL
Instruction Manual	1	1	1

Storage and Stability

All components except RNA-Solv Reagent in Total RNA Kit II should be stored at room temperature. RNA-Solv Reagent should be store at 2-8 ºC for long term storage. All Total RNA Kit II components are guaranteed for at least 12 months from the date of purchase when stored at 22-25°C.

Preparing Reagents

Dilute RNA Wash Buffer II with 100% ethanol as follows and stored at room temperature.

Kit	Ethanol to be Added
R6934-00	20 mL
R6934-01	48 mL
R6934-02	200 mL

- Please remember to always wear gloves whenever working with RNA. This will minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.

- To freeze tissue for long term storage, flash-frozen liquid nitrogen and immediately transfer to -70°C. Tissue can be store for up to 6 months at -70°C. To process the sample, do not thaw the sample during weighing or handing prior to the disruption with RNA-Solv Reagent. Homogenized tissue lysates can be store at -70°C for at least 6 months. To proceed with the frozen tissue lysates, thaw the sample at 37°C until they are completely thawed and all salts in the lysis buffer are dissolved. Do not extend the treatment in 37°C because it can cause chemical degradation of RNA.

- It is very important to determine the correct amount of starting material before the experiment. If the maximum amount of starting material is 100 mg. The capacity of the HiBind® RNA Mini Column is 100 µg. For samples containing high amounts of RNA, we suggest using 30 mg of tissue to start. For samples containing lower level RNA, the maximum amount of starting material (100 mg) can be used.

Recommended Settings

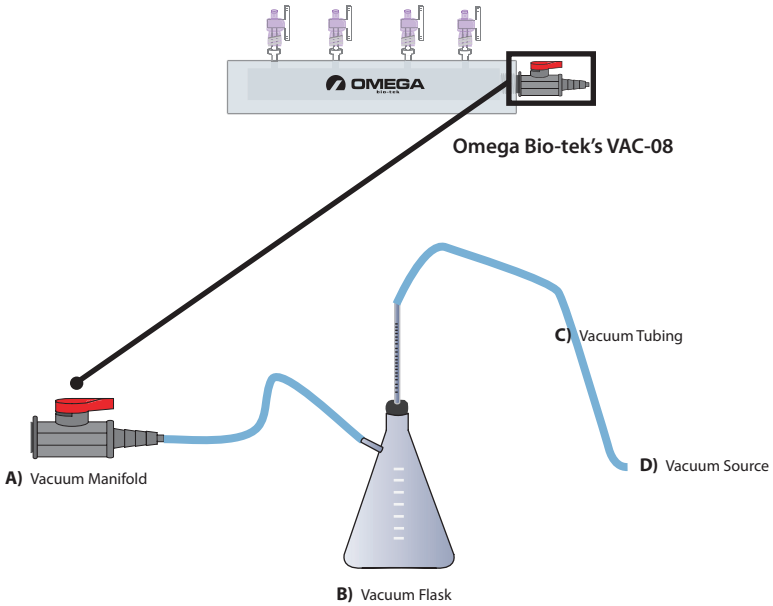
The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-08)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Manifold	Recommended Pressure (mbar)
VAC-08	-200 to -600

Conversion from millibars:	Multiply by:
Millimeters of mercury (mm Hg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Illustrated Vacuum Setup:



Quantification of RNA

Storage of RNA

Purified RNA can be stored at -70°C (RNase-free water). Under such conditions, RNA prepared with the E.Z.N.A.® Total RNA Kit I is stable for more than a year.

Quantification of RNA

To determine the concentration and purity of RNA, one should measure the absorbance at 260 nm and 280 nm in a spectrophotometer. One O.D. unit measured at 260 nm corresponds to 40 µg RNA per mL. DEPC Water is slightly acidic and can dramatically lower absorbance values. We suggest that you dilute the sample in a buffered solution (TE) for spectrophotometric analysis. The A_{260}/A_{280} ratio of pure nucleic acids is 2.0, while for pure protein is approximately 0.6. Therefore, a ratio of 1.8-2.0 corresponds to 90-100% pure nucleic acid. Phenol has a maximum absorbance at 275 nm and can interfere with absorbance readings of DNA or RNA. However, the E.Z.N.A.® Total RNA Kit eliminates the use of phenol and avoids this problem.

RNA Quality

It is highly recommended that RNA Quality be determined prior to all analysis. The quality of RNA can be best assessed by denaturing agarose gel electrophoresis and ethidium bromide staining. Two sharp bands should appear on the gel. These are the 28S and the 18S (23S and 16S for bacteria) ribosomal RNA bands. If these band appears as a smear towards lower molecular weight sized RNAs, the it is likely that RNA has undergone major degradation during preparation, handling, or storage. Although RNA molecules less than 200 bases in length do not efficiently bind to the HiBind® matrix, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

Expected Yields

For animal cell yields, see page 9.

For animal tissue yields, see page 14.

Disruption and Homogenization of Samples

Efficient sample disruption and homogenization is essential for successful Total RNA isolation. Complete cell wall and plasma membrane disruption is very important for the release of all of the RNA contained in the sample. The purpose of homogenization is to reduce the viscosity of the cell lysates produced by cell disruption. Homogenization shears genomic DNA and other high molecular weight cell components thereby creating a homogenous lysate. Incomplete homogenization will cause RNA binding to clog thus preventing efficient RNA binding result in low or no yield.

Mortar and Pestle: Sample Disruption
Sample disruption using a mortar and pestle followed the chosen of homogenization method:

Wear gloves, and take great care when working with liquid nitrogen.

1. Excise tissue and promptly freeze in a small volume of liquid nitrogen.

2. Grind tissue with a ceramic mortar and pestle under approximately 10 mL liquid nitrogen. Pour the suspension into a pre-cooled 15 mL polypropylene tube. The tube must be pre-cooled in liquid nitrogen or the suspension will boil vigorously possibly causing tissue loss.

3. Allow the liquid nitrogen to completely evaporate and add RNA-Solv Reagent.

Homogenization:

A) Homogenizer Spin column (Product # HCR 003)
Load the lysate into a homogenizer spin column pre-inserted into a 2 mL Collection Tube. Spin for two minutes at maximum speed in a microcentrifuge in order to collect homogenized lysate.

B) Syringe and Needle
Shear high molecular-weight DNA by passing the lysate through a narrow needle (19-21 gauge) 5-10 times.

Rotor-Stator Homogenizer: Sample Disruption and Homogenization

Using a rotor-stator homogenizer for sample disruption and homogenization can simultaneously disrupt and homogenize most samples. The process usually takes less than a minute depending on sample type. Many Rotor-stator homogenizers operate with differently sized probes or generators that allow sample processing in 50 mL tubes.

Bead Milling: Sample Disruption and Homogenization

By using bead milling, cells and tissue can be disrupted and homogenized by rapid agitation in the presence of glass beads and a lysis buffer. The optimal amount of glass beads to use for RNA isolation are 0.5 mm for yeast/unicellular cells and 4-8 mm for animal tissue samples.

E.Z.N.A.® Total RNA Kit II Animal Cell Protocol

All centrifugation steps used are preformed at room temperature.

General Protocol Equipment:

- 2-mercaptoethanol
- Chloroform
- Centrifuge with capable of at least 12,000 x *g* and 4°C
- 70% ethanol in DEPC-treated sterile distilled water
- Sterile RNase-free pipette tips and 1.5ml centrifuge tubes
- 100% Ethanol

Sample Disruption and Homogenization Equipment:

- Omega Homogenizer Columns (Cat# HCR003)
- Needle and Syringe
- Mortar and pestle
- Glass Beads
- Rotor-stator Homogenizer

1. Determine the proper amount of starting material: It is critical to use the correct number of starting cells in order to obtain optimal yield and purity with the HiBind® RNA Mini Column. The maximum number of cells that can be processed on a HiBind® RNA Mini Column is dependent on the specific RNA contents and type of cell line. The maximum binding capacity of the HiBind® RNA Mini Column is 100 µg. The maximum number of cells that RNA-Solv Reagent can use in the Total RNA Protocol is 1 x 10^7. Use the following table as a guideline to select the correct starting material.

Expected Yield

Source	Number of Cells	RNA Yield (µg)
IC21	1 x 10^6	12
Hela	1 x 10^6	15
293HEK	1 x 10^6	10
HIN3T3	1 x 10^6	15

2. **Harvest cells by choosing one of the following methods (A or B). (do not use more than 1 x 10^7 cells)**

   A) For cells grown in suspension: determine the number of cells. Pellet the appropriate number of cells by centrifuging at 500 x *g* for 5 minutes. Aspirate the supernatant and continue with step 3 of this protocol.

   Or

   B) For cells grown in a monolayer: These cells can either be lysed directly in the cell culture dish or trypsinized and collected as a cell pellet prior to lysis. Cells grown in cell-culture flasks should always be trypsinized.

   i. **For direct cell lysis:**
   Determine cell number, and aspirate the cell-culture medium completely. Immediately proceed to step 3.

   Note: Not removing cell-culture medium completely will inhibit lysis and dilute the lysate. This will affect the conditions for binding of RNA to the HiBind® RNA Mini Column, and may reduce RNA yield.

   ii. **To trypsinize and collect cells:**
   Determine cell number. Aspirate the medium and wash cells with PBS. Aspirate the PBS, add 0.1-.25% Trypsin into PBS. Add medium (containing serum to inactivate the tryspin), after the cells detach from the dish or flask. Transfer cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 500 x g for 5 minutes. Aspirate the supernatant completely, and proceed to step 3.

   Note: Not removing cell-culture medium completely will inhibit lysis and dilute the lysate. This will affect the conditions for binding of RNA to the HiBind® RNA Mini Column, and may reduce RNA yield.

3. Disrupt cells (do not use more than 1 x 10^7 cells) with RNA-Solv Reagent. For pelleted cells, loosen the cell pellet thoroughly by flicking the tube and then add the appropriate amount of RNA-Solv Reagent based on the table below. To directly lyse the cells in the culture dish, add the appropriate amount of RNA-Solv Reagent directly to the dish. Remember to add 20 µL 2-mercaptoethanol per 1 mL RNA-Solv Reagent before use.

Number of Cells	RNA-Solv Reagent
< 5 x 10^6	500 µL
5 x 10^6 - 1 x 10^7	1000 µL

4. Disrupt and homogenize the sample by using one of the methods described on page 8.

5. Incubate the tube containing the homogenate at room temperature for 5 minutes.

6. Add 100 µL (for < 5 x 10^6 cells) or 200 µL (for 5 x 10^6 - 1 x 10^7 cells) chloroform to the homogenate, close the cap of the tube and vortex for 20 seconds. Incubate at room temperature for 2-3 minutes.

7. Centrifuge at ≥12,000 x *g* at 4°C for 15 minutes to separate the aqueous and organic phase.

   Note: The sample should be separated into 3 phases: an upper colorless aqueous phase, which contains RNA; a white inter phase and a lower blue organic phase.

8. Transfer the upper aqueous phase (around 250 µL or 500 µL) into a new 1.5 mL microcentrifuge tube. Add an equal volume of 70% ethanol and vortex to mix thoroughly. A precipitate may form at this point. This will not interfere with the RNA purification.

9. Apply the sample to a HiBind® RNA Mini Column inserted into a 2 mL Collection Tube. The maximum capacity of the spin column is 700 µL. (Larger volumes can be loaded successively to the column). Centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the filtrate and reuse the collection tube in the next step.

10. Add 500 µL RNA Wash Buffer I directly onto the HiBind® RNA Mini Column. Centrifuge at 10,000 x *g* for 30 seconds. Discard the filtrate and collection tube.

    Note: This the starting point for the OPTIONAL on-membrane DNase I Digestion Protocol (Page 17).

11. Place the column in a new 2 mL Collection Tube and add 500 µL RNA Wash Buffer II. Centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the filtrate and reuse the collection tube in the next step.

    Note: RNA Wash Buffer II must be diluted with ethanol before use. Refer to label on bottle for directions.

12. Add 500 µL RNA Wash Buffer II to the HiBind® RNA Mini Column. Centrifuge at 10,000 x *g* for 1 minute and discard the filtrate. Then with the collection tube empty, centrifuge the HiBind® RNA Mini Column for 2 minutes at maximum speed to completely dry the HiBind® matrix.

13. Transfer the column to a clean 1.5 mL microcentrifuge tube (not supplied) and elute the RNA with 45-75 µL DEPC Water. Make sure to add water directly onto the center of the column matrix. Centrifuge 1 minute at 10,000 x *g*. A second elution may be necessary if the expected yield of RNA >30 µg.

    Alternatively, RNA may be eluted with a greater volume of water. While additional elutions increase total RNA yield, the concentration will be lowered since more than 80% of RNA is recovered with the first elution. Pre-heating the water to 70°C before adding to column and incubating column for 5 minutes at room temperature before centrifugation may increase yields.

E.Z.N.A.® Total RNA Kit II Animal Tissue Protocol

All centrifugation steps used are preformed at room temperature.

General Protocol Equipment:

- 2-mercaptoethanol
- Chloroform
- Microcentrifuge capable of at least 12,000 x g and 4°C
- 70% ethanol in DEPC-treated sterile distilled water
- Sterile RNase-free pipette tips and 1.5ml centrifuge tubes
- 100% Ethanol

Sample Disruption and Homogenization Equipment:

- Liquid Nitrogen
- Omega Homogenizer Columns (Cat# HCR003)
- Needle and Syringe
- Mortar and pestle
- Glass Beads
- Rotor-stator Homogenizer

1. Determine the proper amount of starting material: It is critical to use the correct amount of tissue to obtain optimal yield and purity with the HiBind® RNA Mini Column. The maximum amount of tissue that can be processed on a HiBind® RNA column varies depending on the specific RNA contents and type of tissue. The Maximum binding capacity of the HiBind® RNA column is 100 µg. The maximum amount of tissue that can be used with RNA-Solv Reagent is 30 mg. Use the following table as a guideline to select the correct amount of starting material. If you have no information about your starting material, use 10 mg as a starting amount, based upon the yield and quality of RNA obtained from 10 mg, adjust the starting amount in the next purification.

Average Yield of Total Cellular RNA From Mouse Tissue

Source	Amount of Tissue (mg)	RNA Yield (µg)
Sample Type : Mouse Tissue		
Brain	10	10
Kidney	10	30
Liver	10	45
Heart	10	5
Spleen	10	33
Lung	10	12
Pancreas	10	40
Thymus	10	20

2. Disrupt Tissue and homogenize the tissue in 1 mL RNA-Solv Reagent using one of the described methods on Page 4. (Do not use more than 30 mg tissue). Remember to add 20 µL 2-mercaptoethanol per 1 mL RNA-Solv Reagent before use.

   Note: Incomplete homogenization of the sample will cause lower yields and clogging of the column. It is recommended to homogenize the sample with rotor-stator homogenizers since it normally produce better yield.

3. Incubate the tube containing the homogenate at room temperature for 5 minutes.

4. Add 200 µL chloroform to the homogenate, close the cap of the tube and vortex for 20 seconds. Incubate at room temperature for 2-3 minutes.

5. Centrifuge at ≥12,000 x *g* at 4°C for 15 minutes to separate the aqueous and organic phase.

   Note: The sample should be separated into 3 phases: an upper colorless aqueous phase, which contains RNA; a white inter phase and a lower blue organic phase.

6. Transfer the upper aqueous phase (around 500 µL) into a new 1.5 mL microcentrifuge tube. Add an equal volume of 70% ethanol and vortex to mix thoroughly. A precipitate may form at this point. This will not interfere with the RNA purification.

7. Apply sample onto HiBind® RNA Mini Column inserted into a 2 mL Collection Tube. The maximum capacity of the HiBind® RNA Mini Column is 700 µL. (Larger volumes can be loaded successively.) Centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the flow-through and re-use the collection tube in the next step.

8. Add 500 µL RNA Wash Buffer I by pipetting directly into the spin column. Centrifuge at 10,000 x *g* for 1 minute at room temperature then discard the 2 mL Collection Tube and the filtrate.

   Note: This the starting point for the OPTIONAL on-membrane DNase I Digestion Protocol (Page 17).

9. Place the column into a clean 2 mL Collection Tube and add 500 µL RNA Wash Buffer II. Centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the filtrate and reuse the collection tube in the next step.

   Note: RNA Wash Buffer II must be diluted with ethanol before use. Refer to label on bottle for directions.

10. Add a second 500 µL Wash Buffer II to the HiBind® RNA Mini Column. Centrifuge at 10,000 x *g* for 1 minute and discard the filtrate.

11. Then with the HiBind® RNA Mini Column and collection tube emptied, centrifuge the spin cartridge for 2 minutes at maximum speed to completely dry the HiBind® matrix.

12. Transfer the column to a clean 1.5 mL microcentrifuge tube (not supplied) and elute the RNA with 45-70 µL DEPC Water. Make sure to add water directly onto the center of the column matrix. Centrifuge for 2 minutes at 10,000 x *g*. A second elution may be necessary if the expected yield of RNA > 30 µg.

    Alternatively, RNA may be eluted with a greater volume of water. While additional elutions increase total RNA yield, the concentration will be lowered since more than 80% of RNA is recovered with the first elution. Pre-heating the water to 70°C before adding to column and incubating the column for 5 minutes at room temperature before centrifugation may increase yields.

E.Z.N.A.® Total RNA Kit Vacuum/Spin Protocol

Carry out lysis, homogenization steps as indicated in previous protocols. Instead of continuing with centrifugation, follow the steps below. Do not use more than $1x10^6$ cells or 10 mg tissue for the vacuum protocol.

Note: Please read through previous section of this manual before proceeding with this protocol.

User Supplied Equipment:

- Vacuum manifold
- Vacuum source
- 100% Ethanol

Things to do before starting:

- Assemble vacuum manifold (see Page 6)

1. Prepare the vacuum manifold according to manufacturer's instruction and connect the HiBind® RNA Mini Column to the manifold.

2. Transfer the upper aqueous phase (around 250 µL or 500 µL) into a new 1.5 mL microcentrifuge tube. Add an equal volume of 70% ethanol and vortex to mix thoroughly. A precipitate may form at this point. This will not interfere with RNA purification.

3. Load the homogenized sample onto HiBind® RNA Mini Column.

4. Switch on vacuum source to draw the sample through the column and turn off the vacuum.

5. Add 300 µL RNA Wash Buffer I and draw the wash buffer through the column by turning on the vacuum source.

   Note: This the starting point for the OPTIONAL on-membrane DNase I Digestion Protocol (Page 17).

6. Add 500 µL RNA Wash Buffer I and draw the wash buffer through the column by turning on the vacuum source.

7. Add 700 µL RNA Wash Buffer II and draw the wash buffer through the column by turning on the vacuum source.

   Note: RNA Wash Buffer II must be diluted with absolute ethanol before use. Refer to label for instructions. If refrigerated, RNA Wash Buffer II must be brought to room temperature before use.

8. Assemble the column into a 2 mL Collection Tube and transfer the column to a microcentrifuge tube. Add 200 µL RNA Wash Buffer II into the column. Centrifuge at 10,000 x *g* for 1 minute and discard the filtrate.

9. Then with the HiBind® RNA Mini Column and collection tube emptied, centrifuge the spin cartridge for 2 minutes at maximum speed to completely dry the HiBind® matrix.

10. Place the column in a clean 1.5 mL microcentrifuge tube and add 50-100 µL DEPC Water. Stand for 1-2 minutes and centrifuge for 1 minute to elute the RNA.

E.Z.N.A.® Total RNA Kit II DNase I Digestion Protocol

For most downstream applications it is not necessary to do DNase I digestion due to HiBind® matrix and spin column technology removing nearly all DNA without the need for DNase Treatment. However, certain sensitive RNA applications might require further removal of DNA. In such case, we recommend that you please follow the outlined steps below using product E1091.

Note: After completing steps 1-5 of either of the centrifugation protocols or steps 1-2 of the vacuum protocol (making sure that all of the sample has completely passed through the HiBind® RNA Mini Column), proceed with the following steps.

All centrifugation steps used are performed at room temperature.

User Supplied Material:

- RNase Free DNase Set (Cat# E1091)

1. For each HiBind® RNA Mini Column, prepare the DNase I stock solution as follows:

Buffer	Volume per Prep
E.Z.N.A.® DNase I Digestion Buffer	73.5 µL
RNase-free DNase I (20 Kunitz/µL)	1.5 µL
Total Volume	75 µL

Note:

- DNase I is very sensitive to physical denaturation, therefore do not vortex this DNase I mixture. Please mix by GENTLY inverting the tube. Remember to freshly prepare DNase I stock solution right before RNA isolation.
- E.Z.N.A.® DNase I Digestion Buffer is supplied with Omega Bio-tek, Inc.'s RNase-free DNase Set (Cat# E1091). Standard DNase buffers are not compatible with on-membrane DNase I digestion. The use of other buffers may affect the binding of RNA to the HiBind® matrix, reducing RNA yields, and purity.

2. Add 250 µL RNA Wash Buffer I by pipetting directly onto a new HiBind® RNA Mini Column inserted in a 2 mL Collection Tube. Centrifuge at 10,000 x *g* for 60 seconds and discard the filtrate and collection tube.

Optional DNase I Digestion Protocol

3. Place the HiBind® RNA Mini Column into a new 2 mL Collection Tube. Pipet 75 µL DNase I stock solution directly onto the surface of the HiBind® matrix in each column. Make sure to pipet the stock solution directly onto the center of membrane. DNase I digestion will not go through completion if some of the stock solution remains stuck to the wall or the o-ring of the HiBind® RNA Mini Column.

4. Incubate at room temperature for 15 minutes.

5. Add 500 µL RNA Wash Buffer I. Place the column on a bench top for 2 minutes. Centrifuge at 10,000 x *g* for 60 seconds and discard filtrate and reuse the collection tube.

6. Add 500 µL RNA Wash Buffer II. Centrifuge at 10,000 x *g* for 60 seconds and discard filtrate and reuse the collection tube

   Important: RNA Wash Buffer II must be diluted with ethanol before use. Refer to label for instructions. If refrigerated, RNA Wash Buffer II must be brought to room temperature before use.

7. Add another 500 µL of RNA Wash Buffer II. Centrifuge at 10,000 x *g* for 60 seconds and discard filtrate and reuse the collection tube.

8. With the empty collection tube centrifuge the HiBind® matrix for 2 minutes at maximum speed to completely dry the HiBind® matrix.

9. Place the column in a clean 1.5 mL microcentrifuge tube (not supplied), and add 40-70 µL of DEPC Water. Make sure to add water directly onto the center of the column matrix. Let it sit for 1 minute, and then centrifuge for 2 minutes at maximum speed to elute the RNA. A second elution may be necessary if the expected yield of RNA > 30µg.

   Alternatively, RNA may be eluted with a greater volume of water. While additional elutions increase total RNA yield, the concentration will be lower since more than 80% of RNA has been recovered in the first elution.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **(800-832-8896).**

Possible Problems and Suggestions

Problem	Cause	Solution
Little or no RNA eluted	RNA remains on the column	Repeat elution step.
	Column is overloaded	Reduce quantity of starting material.
Clogged column	Incomplete homogenization	Completely homogenize sample.
		Increase centrifugation time.
		Reduce amount of starting material
Degraded RNA	Starting Culture Problems	Freeze starting material quickly in liquid nitrogen
		Do not store tissue culture cells prior to extraction unless they are lysed first.
		Follow protocol closely, and work quickly.
	RNase contamination	Ensure not to introduce RNase during the procedure.
		Check buffers for RNase contamination.
Problem in downstream applications	Salt carry-over during elution	Ensure RNA Wash Buffer II has been diluted with 4 volumes of ethanol as indicated on bottle.
		RNA Wash Buffer II must be stored and used at room temperature.
		Repeat wash with RNA Wash Buffer II.
DNA contamination	DNA contamination	Digest with RNase-free DNase and inactivate DNase by incubate at 65°C for 5 minutes in the presence of EDTA.
Low Abs ratios	RNA diluted in acidic buffer or water	DEPC Water is acidic and can dramatically lower Abs_{260} values. Use TE buffer to dilute RNA prior to spectrophotometric analysis.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
TRK Lysis Buffer (100 mL)	PR021
RNA Wash Buffer (100 mL)	PR030
RNA Wash Buffer II (25 mL)	PR031
DEPC Water (100 mL)	PR032
2ml Capless collection tubes	SS1-1370-00
1.5ml DNase/RNase Free Centrifuge Tubes	SS1-1210-00
RNase-free DNase Set (5o preps)	E1091
RNase-free DNase Set (200 preps)	E1091-02
Omega Homogenizer Columns (200 preps)	HCR003
Proteinase K Solution	AC115-AC116