E.Z.N.A.® Total RNA Kit I

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OMEGA bio-tek

Innovations in nucleic acid isolation

Introduction

The E.Z.N.A.® RNA family of products is an innovative system that radically simplifies the extraction and purification of RNA from a variety of sources. The key to this system is that it uses the reversible binding properties of the HiBind Matrix in combination with the speed of mini-column spin technology thereby permitting single or multiple simultaneous processing of samples. There is no need for phenol/chloroform extractions and time-consuming steps such as CsCl gradient ultracentrifugation, or precipitation with isopropanol or LiCl are eliminated. RNA purified using the E.Z.N.A.® RNA Purification System 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 I can purify up to 100 µg of total RNA from cultured eukaryotic cells or tissue. Normally, 1 x 10^6 - 1 x 10^7 eukaryotic cells, or 5-30 mg of tissue, can be processed in a single experiment. Fresh, frozen or RNALater® stabilized tissues can be used. Lysis of cells or tissue occurs under denaturing conditions which inactivate RNases. After the homogenization process, samples are applied to the HiBind RNA spin column to which total RNA binds. Cellular debris and other contaminants are effectively washed away after a few quick wash steps. High quality RNA is finally eluted in DEPC treated water. Total RNA greater than 200 nt is isolated using this kit.

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 (Product # R6814) as it is specifically designed for effective hemolysis and hemoglobin removal, thereby giving higher RNA yields.

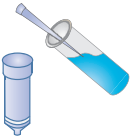
Binding Capacity

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

Centrifugation Protocols

Add TRK Buffer and Lyse

Homogenize



Add Ethanol

Apply Sample to Column

Wash 3x

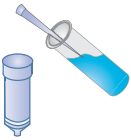
Dry

Elute

Vacuum Protocol

Add TRK Buffer and Lyse

Homogenize



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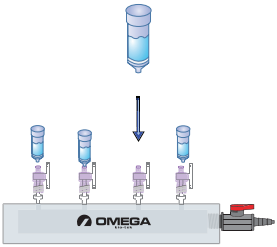
Apply Sample to Column



Wash 3x

Dry

Elute

Kit Contents

Total RNA Kit I	R6834-00	R6834-01	R6834-02
Preparations	5	50	200
HiBind RNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
TRK Lysis Buffer	5 mL	40 mL	150 mL
RNA Wash Buffer I	5 mL	50 mL	200 mL
RNA Wash Buffer II	5 mL	12 mL	50 mL
DEPC Water	1 mL	10 mL	40 mL
Instruction Booklet	1	1	1

Storage and Stability

All E.Z.N.A.® Total RNA Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature. During shipment, crystals or precipitation may form in the TRK Lysis Buffer. Dissolve by warming buffer to 37°C.

- Dilute RNA Wash Buffer II with absolute ethanol (96-100%) as follows:

Kit	Ethanol To Be Added
R6834-00	Add 20 mL absolute ethanol
R6834-01	Add 48 mL absolute ethanol
R6834-02	Add 200 mL absolute ethanol

- Store diluted RNA Wash Buffer II at room temperature.

- 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.

- Under cool ambient conditions, crystals may form in TRK Lysis Buffer. This is normal; warm at 37°C to dissolve.

- **Optional:** As a preparation step add 20µl of 2-mercaptoethanol (β-mercaptoethanol) per 1 ml of TRK Lysis Buffer in order to achieve a working solution. This mixture can be stored for 1 week at room temperature.

Guideline for Vacuum Manifold

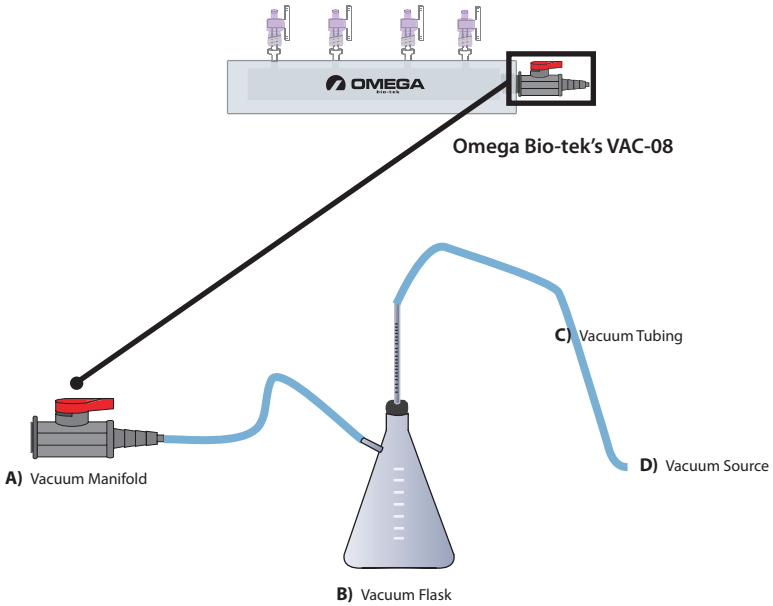
The following is required for use with the Vacuum Protocol:

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. 1 O.D. unit measured at 260 nm corresponds to 40µg of RNA per ml. DEPC treated 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 of liquid nitrogen. Pour the suspension into a pre-cooled 15ml 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 TRK lysis buffer.

Homogenization:

A) Homogenizer Spin column (Product # HCR 003)
Load the lysate into a homogenizer spin column pre-inserted into a 2ml collection tube. Spin for two minutes at maximum speed in a micro centrifuge 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 50ml 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 0.5mm for yeast/unicellular cells and 4-8mm for animal tissue samples.

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

All centrifugation steps used are preformed at room temperature.

General Protocol Equipment:

- Absolute(~96-100%) Ethanol
- Sterile RNase-free pipet tips and 1.5ml centrifuge tubes
- **Optional:** 14.3M β-mercaptoethanol (β-ME, 2-mercaptoethanol)
- Microcentrifuge capable of at least 14,000 x g
- 70% ethanol in Sterile DEPC-treated Water.
- Disposable gloves

Sample Disruption and Homogenization Equipment:

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

Things to do before starting:

- Optional: As a preparation step add 20µl of 2-mercaptoethanol (β-mercaptoethanol) per 1 ml of TRK Lysis Buffer in order to achieve a working solution. This mixture can be stored for 1 week at room temperature.

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 column. The maximum number of cells that can be processed on a HiBind RNA column is dependent on the specific RNA contents and type of cell line. The maximum binding capacity of the HiBind RNA column is 100µg. The maximum number of cells that TRK Lysis Buffer can use in the Total RNA Protocol is 1×10^7. Use the following table as a guideline to select the correct starting material.

Expected Yield

Source	Number of Cells	RNA Yield (µg)
IC21	**1×10^6**	**12**
Hela	**1×10^6**	**15**
293HEK	**1×10^6**	**10**
HIN3T3	**1×10^6**	**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 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 column, and may reduce RNA yield.

3. Disrupt cells (do not use more than 1 x 10^7 cells) with TRK Lysis Buffer.

For pelleted cells, loosen the cell pellet thoroughly by flicking the tube, and adding the appropriate amount of TRK Lysis Buffer based on the table below. Mix throughly by vortexing or pipetting.

Number of Cells	Amount of TRK Lysis Buffer (µl)
< 5 x 10^6	350 **µl**
5 x 10^6 - 1 x 10^7	700 **µl**

For direct lysis of cells grown in a monolayer, add the appropriate amount of TRK Lysis Buffer directly to the cell culture dish based on the table below. Collect the cell lysate with a rubber policemen and transfer the cell lysate into a 1.5 ml centrifuge tube. Mix throughly by vortexing or pipetting. Make sure there is no visible cell clumps in the sample.

Dish diameter (cm)	Amount of TRK Lysis Buffer (µl)
< 6	350 **µl**
6-10	700 **µl**

4. Homogenize and disrupt the tissue accordingly to one of the following steps:

   1. **Rotor-Stator Homogenizer:** Homogenize cells with a rotor-stator homogenizer or until the sample is uniformly homogenized. See Page 8 for details.

   2. **Syringe and Needle: S**hear high molecular-weight DNA by passing the lysate through a narrow needle (19-21 gauge) 5-10 times.

   3. **Omega Homogenizer Column (HCR003):** Load the lysate into a homogenizer spin column pre-inserted into a 2ml collection tube. Spin for two minutes at maximum speed in a micro centrifuge in order to collect homogenized lysate.

   Note: Incomplete homogenization of the sample will cause clogging colum thus resulting lower yields or no yield. It is recommended to homogenize the tissue sample with rotor-stator homogenizers since it normally produces better yields.

5. Add an equal volume (350µl or 700µl) of 70% ethanol to the lysate and mix thoroughly by pipetting up and down 3-5 times. Do not centrifuge. If any sample has lost its volume during homogenization, adjust the volume of ethanol accordingly.

   Note: During RNA purification from certain cell lines, a precipitate may form after the addition of ethanol. This does not affect the procedure.

6. Apply the sample (including any precipitate that may have formed) to a HiBind RNA column inserted into a 2ml collection tube (supplied). Centrifuge at 10,000 x g for 60 seconds at room temperature. Discard flow-through and reuse the collection tube in the next step.

   Note: The maximum capacity of the HiBind RNA spin column is 700µl. Larger volumes can be loaded on to the column successively in the same HiBind RNA spin column. Discard flow-through after each centrifugation.

   Optional: This is the starting point if performing the optional on-column DNase I digestion . Follow protocol as outlined on page 18, after completing this step.

7. Add 500µl of RNA Wash Buffer I by pipetting directly onto the HiBind RNA column. Centrifuge at 10,000 x g for 60 seconds. Discard the flow-through and reuse the collection tube in the next step.

8. Add 500µl of RNA Wash Buffer II by pipetting directly onto the HiBind RNA column. Centrifuge at 10,000 x g for 60 seconds at room temperature. Discard flow-through and reuse the collection tube in the next step.

   Important: 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.

9. Add 500µl of RNA Wash Buffer II by pipetting directly onto the HiBind RNA column. Centrifuge at 10,000 x g for 60 seconds at room temperature and discard flow-through and place the HiBind RNA column in a new 2 mL collection tube(supplied).

10. Centrifuge the HiBind RNA column for 2 minutes at maximum speed to completely dry the HiBind matrix.

11. Elution of RNA: Transfer the column into a clean 1.5 ml centrifuge tube (not supplied), and elute the RNA by adding 40-70µl of DEPC-treated water (supplied). Make sure that you add the water directly onto the center of the column matrix. Centrifuge for 1 minute at maximum speed. A second elution may be necessary if the expected yield of RNA is > 30 µg.

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

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

All centrifugation steps used are preformed at room temperature.

General Protocol Equipment:

- Absolute(~96-100%) Ethanol
- Sterile RNase-free pipet tips and 1.5ml centrifuge tubes
- Optional: 14.3M β-mercaptoethanol (β-ME, 2-mercaptoethanol)
- Microcentrifuge capable of at least 14,000 x g
- 70% ethanol in Sterile DEPC-treated Water.
- Disposable latex gloves

Sample Disruption and Homogenization Equipment:

- Liquid Nitrogen
- Omega Homogenizer Columns (HCR 003)
- Needle and Syringe
- Mortar and pestle
- Glass Beads
- Rotor-stator Homogenizer

Before starting:

- **Optional:** As a preparation step add 20µl of 2-mercaptoethanol β-mercaptoethanol)per 1 ml of TRK Lysis Buffer in order to achieve a working solution. This mixture can be stored for 1 week at room temperature.

1. Determine the proper amount of starting material: It is critical to use the correct tissue amount in order to obtain optimal yield and purity with the HiBind RNA column. The maximum amount of tissue that can be processed on a HiBind RNA column is dependent on the specific RNA contents and type of tissue. The maximum binding capacity of the HiBind RNA column is 100µg. The maximum amount of tissue that TRK Lysis Buffer can lyse in the this protocol is 30mg. Use the following table as a guideline to select the correct starting material. If no information regarding starting material is available, use 10 mg as a starting amount. Given 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**

Amount of TRK Lysis Buffer to be added in step 2 based on the table below. For Samples stored in RNALater® use 700 **µ**L of TRK Lysis Buffer.

Amount of Tissue	Amount of TRK Lysis Buffer (µl)
≤ 15 mg	350 **µl**
20-30 mg	700 **µl**

2. Homogenize and disrupt the tissue accordingly to one of the following steps:

   A. **Rotor-Stator Homogenizer:** Add the appropriate amount of TRK lysis buffer to the sample. Homogenize tissue with a rotor-stator homogenizer or until the sample is uniformly homogenized. (see Page 8 for details)

   B. **Disrupt with Mortar and Pestle then homogenize with one of the following methods :**

   Place the tissue in liquid nitrogen and grind using a mortar and pestle. Transfer the liquid nitrogen and tissue to a 1.5 mL centrifuge tube (not supplied). Let the liquid nitrogen evaporate without allowing the tissue sample to thaw. Add the appropriate amount of TRK lysis buffer to the grounded ample. Then homogenize with one of the following methods:

   I. Transfer the lysate to an Omega Homogenizer column (HCR003) pre-inserted into a 2 mL collection tube. Centrifuge at >13,000 x g for 2 minutes.

   II. Pass the lysate through a narrow needle (19-21 gauge) 5-10 times.

Note: Incomplete homogenization of the sample will cause clogging colum thus resulting lower yields or no yield. It is recommended to homogenize the tissue sample with rotor-stator homogenizers since it normally produces better yield.

3. Centrifuge at full speed (≥ 13,000 x g) for 5 minutes. Carefully transfer the cleared supernatant by pipetting to a clean 1.5 ml centrifuge tube (not supplied). Use only this supernatant (lysate) in subsequent steps.

   Note: In some preparations, a fatty upper layer will form after centrifugation. Transferring any of the fatty upper layer may reduce RNA yields or clog the column.

4. Add an equal volume (350µl or 700µl) of 70% ethanol to the lysate and mix thoroughly by pipetting up and down 3-5 times. Do not centrifuge. If any sample has lost its volume during homogenization, adjust the volume of ethanol accordingly.

   Note: A precipitate may form after the addition of ethanol in certain preparations. This does not affect the procedure.

5. Apply the sample (including any precipitate that may have formed) to a HiBind RNA spin column placed into a 2ml collection tube (supplied). Centrifuge at 10,000 x g for 60 seconds at room temperature. Discard flow-through and reuse the collection tube in the next step.

   Note: The maximum capacity of the HiBind RNA spin column is 700 µl. Larger volumes can be loaded on to the column successively in the same HiBind RNA spin column. Discard flow-through after each centrifugation.

   Optional: This is the starting point if performing the optional on-column DNase I digestion. Follow protocol as outlined on page 18, Immediately after completion of step

6. Add 500 µl of RNA Wash Buffer I by pipetting directly onto the HiBind RNA Column. Centrifuge at 10,000 x g for 30 seconds. Discard the flow-through and reuse the collection tube in the next step.

7. Add 500 µl of RNA Wash Buffer II by pipetting directly onto the HiBind RNA Column. Centrifuge at 10,000 x g for 30 seconds at room temperature. Discard flow-through and reuse the collection tube in the next step.

   Important: 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. Add 500 µl of RNA Wash Buffer II by pipetting directly onto the HiBind RNA Column. Centrifuge at 10,000 x g for 30 seconds at room temperature. Discard flow-through and place the HiBind RNA column in a new 2 mL Collection tube(supplied)..

9. With the empty 2 ml collection tube, centrifuge the HiBind RNA column for 2 minutes at maximum speed to completely dry the HiBind matrix.

10. **Elution of RNA:** Transfer the column into a clean 1.5 ml centrifuge tube (not supplied), and elute the RNA with 40-70µl of DEPC-treated water (supplied). Make sure that you add the water directly onto the center of the column matrix. Centrifuge for 2 minutes at maximum speed (≥ 13,000 xg). A second elution may be necessary if the expected yield of RNA is > 30 µg.

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 10^6 cells or 10 mg of 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

Things to do before starting:
- Assemble vaccum manifold (see page 6)

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

2. Load the homogenized sample onto the HiBInd RNA column.

   Note: Steps 1-4 from the Total RNA Animal Cell protocol should be completed or steps 1-4 from the Total RNA Animal Tissue Protocol should be completed before loading the sample to the HiBind RNA column.

3. Switch on the vacuum source to draw the sample through the column.

4. Add 500 µl of RNA Wash Buffer I, draw the buffer through the column by turning on the vacuum source. Turn off the vacuum source when the buffer has been completely drawn through the column.

5. Add 500 µl of RNA Wash Buffer II, draw the buffer through the column by turning on the vacuum source. Turn off the vacuum source when the buffer has been completely drawn through the column.

   Important: 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.

6. Add another 500 µl of RNA Wash Buffer II, draw the buffer through the column by turning on the vacuum source. Turn off the vacuum source when the buffer has been completely drawn through the column.

7. Assemble the column into a 2 ml collection tube(supplied) and transfer the column to a micro centrifuge. Centrifuge at full speed for 2 minutes to dry the column matrix.

8. Place the column in a clean 1.5 ml microcentrifuge tube (not supplied), and add 40-70µl of DEPC-treated water (supplied). Make sure that you add the 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 is > 30 µg.

Optional DNase Digestion Protocol

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

For most downstream applications it is not necessary to do DNase digestion due to HiBind RNA resin 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 column), proceed with the following steps.

All centrifugation steps used are preformed at room temperature.

User Supplied Material:
- RNase Free DNase Set (E1091)

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

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

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 (product no. E1091). Standard DNase Buffers are not compatible with on-membrane DNase digestion. The use of other buffers may affect the binding of RNA to the HiBind matrix, reducing RNA yields, and purity.

2. **Add 250µl of RNA Wash Buffer I** by pipetting directly onto a new HiBind RNA column inserted in a 2 ml collection tube. Centrifuge at 10,000 x g for 60 seconds and discard the flow-through and collection tube.

Optional DNase Digestion Protocol

3. Place the RNA column into a new 2 ml collection tube. Pipet 75µl of the DNase I stock solution directly onto the surface of the HiBind RNA resin 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 column.

4. Incubate at room temperature (25-30°C) for 15 minutes.

5. Add 500 µl of 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 flow-through and reuse the collection tube.

6. Add 500µl of RNA Wash Buffer II. Centrifuge at 10,000 x g for 60 seconds and discard flow-through and reuse the collection tube

   Important: 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.

7. Add another 500µl of RNA Wash Buffer II. Centrifuge at 10,000 x g for 60 seconds and discard flow-through 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 40-70µl of DEPC-treated water (supplied). 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.
Problem	Cause	Solution
Clogged column	Incomplete homogenization	Completely homogenize sample.
		Increase centrifugation time.
		Reduce amount of starting material
Problem	Cause	Solution
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	Cause	Solution
Problem in downstream applications	Salt carry-over during elution	Ensure Wash Buffer II Concentrate has been diluted with 4 volumes of 100% ethanol as indicated on bottle.
		1 X Wash Buffer II must be stored and used at room temperature.
		Repeat wash with RNA Wash Buffer II.
Problem	Cause	Solution
DNA contamination	DNA contamination	Digest with RNase-free DNase and inactivate DNase by incubate at 65°C for 5 min in the presence of EDTA.
Problem	Cause	Solution
Low Abs ratios	RNA diluted in acidic buffer or water	DEPC-treated 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)

Buffer (Size)	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 (20o preps)	E1091-02
Omega Homogenizer Columns (200 preps)	HCR003
Proteinase K Solution	AC115-AC116

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