

# E.Z.N.A.® HP Total RNA Isolation Kit

## Table of Contents

Introduction.....	2
Illustrated Protocol.....	3
Kit Contents/Storage and Stability.....	4
Preparing Reagents.....	5
Recommended Settings.....	6
Quantification of RNA.....	7
Disruption and Homogenization.....	8
Animal Cell Protocol .....	9
Animal Tissue Protocol .....	13
Spin/Vacuum Manifold Protocol.....	16
DNase Digestion Protocol .....	19
Troubleshooting Guide.....	21
Ordering.....	23

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# Introduction

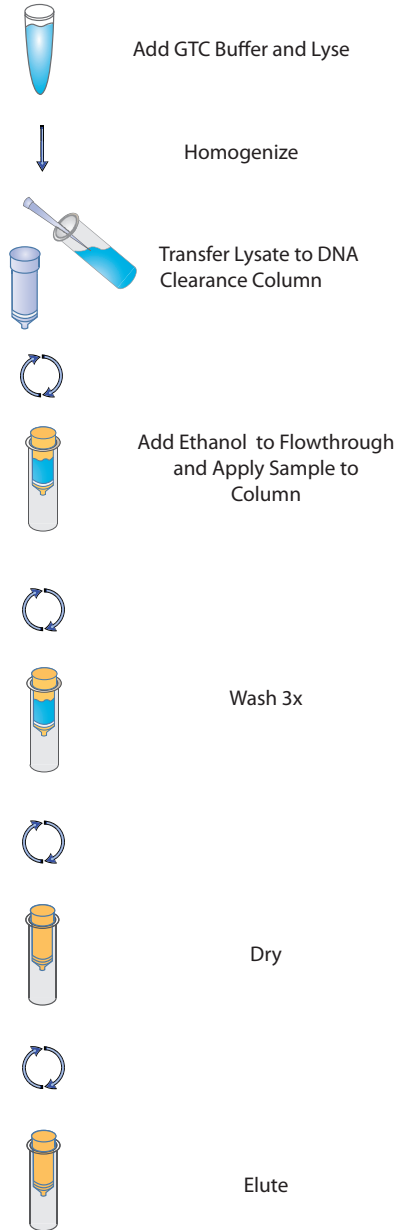
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E.Z.N.A.<sup>™</sup> HP Total RNA Kit provides a rapid and easy method for RNA isolation from a small amount of cultured eukaryotic cells or tissues. This kit allows single or simultaneous processing of multiple samples in less than 40 minutes. Normally,  $1 \times 10^7$  eukaryotic cells or 25-30 mg tissue can be used in a single experiment. There is no need for phenol/chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation, and precipitation with isopropanol or LiCl, are eliminated. RNA purified using the E.Z.N.A.HP Total RNA method is ready for applications such as RT-PCR\*, RT-qPCR, Northern blotting, poly A+ RNA (mRNA) purification, nuclease protection, and in vitro translation.

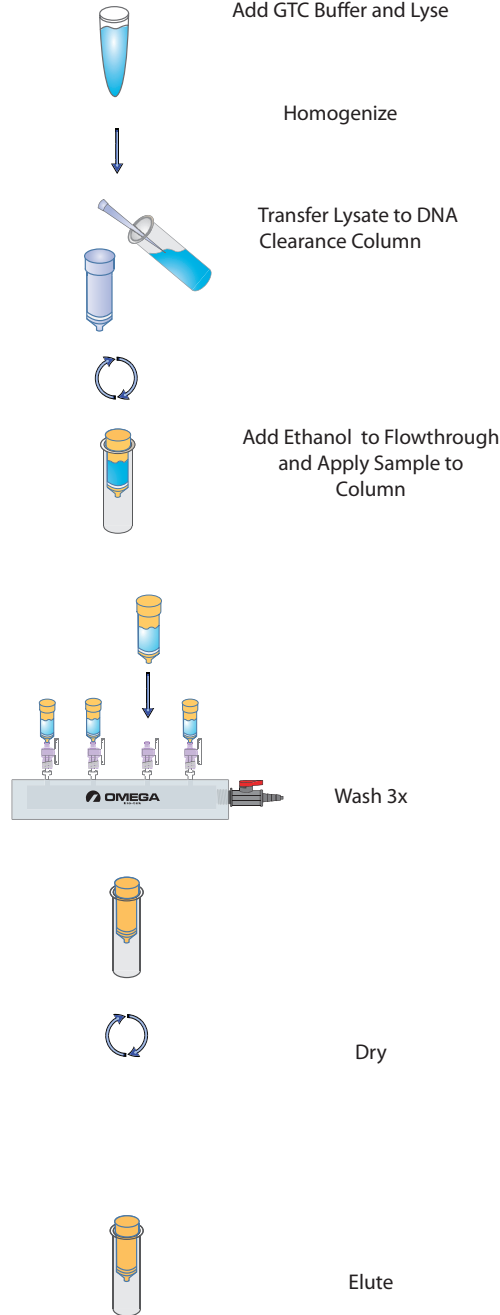
## Principle

The E.Z.N.A.<sup>™</sup> HP Total RNA Kit uses the reversible binding properties of HiBind<sup>®</sup> matrix, a silica-based material. This is combined with the speed of mini-column spin technology. A specifically formulated high salt buffer system allows more than 100µg of RNA fragments greater than 200 bases to bind to the matrix. Cells or tissues are first lysed under denaturing conditions that inactivates RNase. After the homogenization process by either beadmilling or rotor-stator homogenizer, samples are then applied to the HiBind<sup>®</sup> DNA clearance columns to which the genomic DNA binds, the flow-through samples are then applied to a HiBind<sup>®</sup> RNA spin column. After a few quick washing steps which cellular debris and other contaminants are effectively washed away, high quality RNA is eluted in DEPC-treated water.

# Centrifugation Protocols



# Vacuum Protocol



# Kit Contents

HP Total RNA Isolation Kit	R6812-00	R6812-01	R6812-02
Preparations	5	50	200
HiBind RNA Mini Columns	5	50	200
DNA Clearance Columns	5	50	200
2 mL Collection Tubes	15	150	600
GTC 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

E.Z.N.A.™ HP Total RNA Kits should be stored at room temperature. During shipment crystals may form in the GTC Lysis Buffer. Warm to 37°C to dissolve. All E.Z.N.A.™ HP Total RNA Kit components are guaranteed for at least 24 months from the date of purchase when stored at 22-25°C.

\*The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 (and international equivalents) owned by Hoffmann-LaRoche, Inc.

## Before Starting

Please take a few minutes to read this booklet thoroughly and become familiar with the protocol. Prepare all materials required before starting to minimize RNA degradation.

<b>IMPORTANT</b>	<b>Dilute RNA Wash Buffer II with absolute ethanol before use</b>
	R6812-00 Add 20 ml 96-100% ethanol
	R6812-01 Add 48 ml 96-100% ethanol
	R6812-02 Add 200 ml 96-100% ethanol

- 2-mercaptoethanol ( $\beta$ -mercaptoethanol) is key in denaturing RNase and must be added to an aliquot of GTC Lysis Buffer before use.

Add 20  $\mu$ l of 2-mercaptoethanol per 1 ml of GTC Lysis Buffer. This mixture can be stored for 1 week at room temperature.

# Guideline for Vacuum Manifold

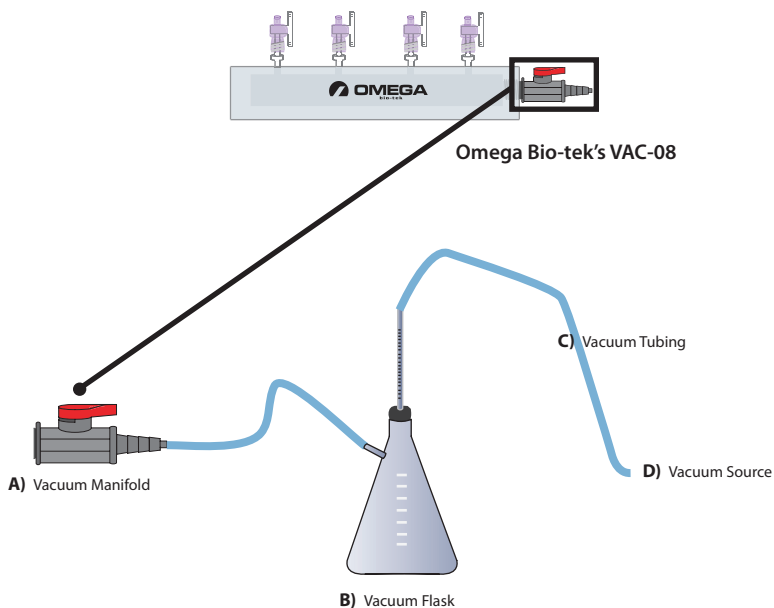
The following is required for use with the Vacuum Protocol:

- A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-08)  
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma Aldrich VM20, 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.® HP Total RNA Isolation Kit 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.® HP Total RNA Isolation 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

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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 GTC 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.



# HP Total RNA Isolation Protocol - Animal Cell

## E.Z.N.A.® HP Total RNA Isolation Kit - 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  $\beta$ -mercaptoethanol ( $\beta$ -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 20 $\mu$ l of 2-mercaptoethanol ( $\beta$ -mercaptoethanol) per 1 ml of GTC Lysis Buffer in order to achieve a working solution. This mixture can be stored for 1 week at room temperature.
  - Prepare RNA Wash Buffer II according to instructions on Page 5
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 100 $\mu$ g. The maximum number of cells that GTC Lysis Buffer can use in the HP Total RNA Protocol is  $1 \times 10^7$ . Use the following table as a guideline to select the correct starting material.

### Expected Yield

Source	Number of Cells	RNA Yield ( $\mu$ g)
IC21	$1 \times 10^6$	12
Hela	$1 \times 10^6$	15
293HEK	$1 \times 10^6$	10
HIN3T3	$1 \times 10^6$	15

# HP Total RNA Isolation Protocol- Animal Cell

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

A) For cells grown in suspension: determine the number of cells. Pellet the appropriate number of cells by centrifuging at  $500 \times g$  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 trypsin), 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  $500 \times g$  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  $1 \times 10^7$  cells) with GTC Lysis Buffer.

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

Number of Cells	Amount of GTC Lysis Buffer ( $\mu$ l)
$< 5 \times 10^6$	350 $\mu$ l
$5 \times 10^6 - 1 \times 10^7$	700 $\mu$ l

# HP Total RNA Isolation Protocol - Animal Cell

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

Dish diameter (cm)	Amount of GTC Lysis Buffer ( $\mu$ l)
< 6	350 $\mu$ l
6-10	700 $\mu$ l

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:** Shear 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 column 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. Transfer the Homogenized Lysate to a DNA Clearance Column Placed in a 2 mL Collection tube. Centrifuge at 13,000 x g for 1 minute. Discard the DNA Clearance Column and Save the flow-through.
6. Add an equal volume (350 $\mu$ l or 700 $\mu$ l) 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.

# HP Total RNA Isolation Protocol - Animal Cell

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7. 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 700 $\mu$ l. 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 19, after completing this step.

8. Add 500 $\mu$ l 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.
9. Add 500 $\mu$ l 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.

10. Add 500 $\mu$ l 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).
11. Centrifuge the HiBind RNA column for 2 minutes at maximum speed to completely dry the HiBind matrix.
12. **Elution of RNA:** Transfer the column into a clean 1.5 ml centrifuge tube (not supplied), and elute the RNA by adding 40-70 $\mu$ l 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  $\mu$ g.

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.

# HP Total RNA Isolation Protocol - Animal Tissue

## E.Z.N.A.® HP Total RNA Isolation Kit - Animal Tissue Protocol

All centrifugation steps used are performed at room temperature.

### General Protocol Equipment:

- Absolute (~96-100%) Ethanol
- Sterile RNase-free pipet tips and 1.5ml centrifuge tubes
- Optional: 14.3M  $\beta$ -mercaptoethanol ( $\beta$ -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 20 $\mu$ l of 2-mercaptoethanol ( $\beta$ -mercaptoethanol) per 1 ml of GTC Lysis Buffer in order to achieve a working solution. This mixture can be stored for 1 week at room temperature.
  - Prepare RNA Wash Buffer II according to instructions on Page 5
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 100 $\mu$ g. The maximum amount of tissue that GTC Lysis Buffer can lyse in 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.

# HP Total RNA Isolation Protocol - Animal Tissue

## Average Yield of Total Cellular RNA From Mouse Tissue

Source	Amount of Tissue (mg)	RNA Yield ( $\mu\text{g}$ )
<i>Sample Type : Mouse Tissue</i>		
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 GTC Lysis Buffer to be added in step 2 based on the table below. For Samples stored in RNALater<sup>®</sup> use 700  $\mu\text{L}$  of GTC Lysis Buffer.

Amount of Tissue	Amount of GTC Lysis Buffer ( $\mu\text{l}$ )
$\leq 15$ mg	350 $\mu\text{l}$
20-30 mg	700 $\mu\text{l}$

2. Homogenize and disrupt the tissue accordingly to one of the following steps:
  - A. **Rotor-Stator Homogenizer:** Add the appropriate amount of GTC 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 GTC 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  $>13,000 \times g$  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.

# HP Total RNA Isolation Protocol- Animal Tissue

3. Centrifuge at full speed ( $\geq 13,000 \times g$ ) for 5 minutes. **Carefully transfer the cleared supernatant by pipetting to a DNA Clearance Column Pre-inserted in a 2 mL Collection Tube**

**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. Centrifuge at  $13,000 \times g$  for 1 minute. Discard the DNA Clearance Column and save the flow-through.

**Note:** Make sure that all of the liquid has passed through the DNA Clearance Column after centrifugation. If necessary repeat the centrifugation until all liquid passes through the membrane.

5. Add an equal volume (350 $\mu$ l or 700 $\mu$ l) 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.

6. 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  $10,000 \times g$  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  $\mu$ l. 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 19, Immediately after completion of step

7. Add 500  $\mu$ l of RNA Wash Buffer I by pipetting directly onto the HiBind RNA Column. Centrifuge at  $10,000 \times g$  for 30 seconds. Discard the flow-through and reuse the collection tube in the next step.
8. Add 500  $\mu$ l of RNA Wash Buffer II by pipetting directly onto the HiBind RNA Column. Centrifuge at  $10,000 \times g$  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.

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

# HP Total RNA Isolation Protocol- Animal Tissue

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10. With the empty 2 ml collection tube, 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 with 40-70 $\mu$ l 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 ( $\geq 13,000$  xg). A second elution may be necessary if the expected yield of RNA is  $> 30$   $\mu$ g.



# HP Total RNA Isolation Kit - Vacuum/Spin Protocol

## E.Z.N.A.<sup>®</sup> HP Total RNA Isolation 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<sup>6</sup> 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 vacuum 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 1-6 from the HP Total RNA Animal Cell Protocol should be completed or steps 1-5 from the HP 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.

## HP Total RNA Isolation Kit - Vacuum/Spin Protocol

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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 40-70 $\mu$ l 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  $\mu$ g.

# Optional DNase Digestion Protocol

## E.Z.N.A.® HP Total RNA Isolation Kit - DNase Digestion Protocol

For most downstream applications it is not necessary to do DNase digestion due to HiBind RNA resin and DNA Clearance technology 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 1-7 of the HP Total RNA Animal Cells Spin Protocol or Steps 1-6 of either of the HP Total RNA Spin Tissue Protocol n 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

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3. Place the RNA column into a new 2 ml collection tube. Pipet 75 $\mu$ l 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  $\mu$ l 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 500 $\mu$ l 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 500 $\mu$ l 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 40-70 $\mu$ l 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 > 30 $\mu$ g.

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.

## Troubleshooting Guide

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.
Low Abs ratios	RNA diluted in acidic buffer or water	DEPC-treated water is acidic and can dramatically lower Abs <sub>260</sub> 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 (50 preps)	E1091
RNase-free DNase Set (200 preps)	E1091-02
Omega Homogenizer Columns (200 preps)	HCR003
Proteinase K Solution	AC115-AC116

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**Notes:**