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

September 2015

For Research Use Only

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E.Z.N.A.® Total RNA Kit II

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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 Nuclease-free 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

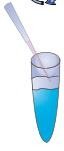
Each HiBind® RNA Mini Column can bind approximately 100 µg RNA. Using greater than 30 mg tissue or 1 x 10^7 cells is not recommended.

Centrifugation Protocols

Add RNA-Solv® Reagent and Lyse

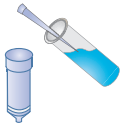
Homogenize

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

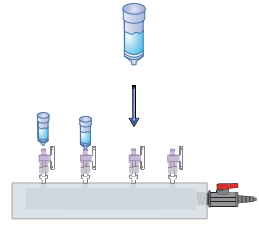
Homogenize

Add Chloroform

Transfer Aqueous Phase

Add Ethanol

Apply Sample to Column



Wash 3x

Dry

Elute

Kit Contents

Product	R6934-00	R6934-01	R6934-02
Purifications	5	50	200
HiBind® RNA Mini Columns	5	50	200
2 mL Collection Tubes	10	100	400
RNA-Solv® Reagent	5 mL	60 mL	220 mL
RNA Wash Buffer I	5 mL	50 mL	200 mL
RNA Wash Buffer II	5 mL	12 mL	50 mL
Nuclease-free Water	5 mL	10 mL	40 mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® Total RNA Kit II components are guaranteed for at least 24 months from the date of purchase when stored as follows. RNA-Solv® Reagent must be stored at 2-8°C. All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in some of the buffers. Dissolve such deposits by warming the solution at 37°C and gently shaking.

Preparing Reagents

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

Kit	100% Ethanol to be Added
R6934-00	20 mL
R6934-01	48 mL
R6934-02	200 mL

- Add 20 µL 2-mercaptoethanol per 1 mL RNA-Solv® Reagent.

- 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-freeze in liquid nitrogen and immediately transfer to -70°C. Tissue can be stored 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 stored 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. The capacity of the HiBind® RNA Mini Column is 100 µg RNA. For samples containing high amounts of RNA, we suggest using 30 mg tissue to start. For samples containing lower level RNA, the amount of starting material can be increased.

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 ll 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. Nuclease-free Water is slightly acidic and can dramatically lower absorbance values. We suggest that you dilute the sample in a buffe red 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 correspond s to 90-100% pure nucleic acid. Phenol has a maximum absorbance at 275 nm and can i nterfere with absorbance readings of DNA or RNA. However, the E.Z.N.A.® Total RNA Kit II 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 8.

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 Mini Column (Product # HCR003)
Load the lysate into a Homogenizer Mini 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 performed at room temperature.

Materials and Equipment to be Supplied by User:

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

Sample Disruption and Homogenization Equipment:

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

Before Starting:

- Prepare RNA Wash Buffer II according to the "Preparing Reagents" section on Page 5.
- Add 20 µL 2-mercaptoethanol per 1 mL RNA-Solv® Reagent.

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 Yields

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 7.

5. Let sit at room temperature for 5 minutes.

6. Add 100 µL (for < 5 x 10^6 cells) or 200 µL (> 5 x 10^6 cells) chloroform. Vortex for 20 seconds to mix thoroughly.

7. Let sit at room temperature for 2-3 minutes.

8. Centrifuge at maximum speed (≥12,000 x *g*) at 4°C for 15 minutes to separate the aqueous and organic phase.

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

9. Transfer the upper aqueous phase (around 250 µL or 500 µL) into a new 1.5 mL microcentrifuge tube.

10. Add an equal volume of 70% ethanol. Vortex to mix thoroughly.

    Note: A precipitate may form at this point. This will not interfere with the RNA purification.

11. Insert a HiBind® RNA Mini Column into a 2 mL Collection Tube.

12. Transfer 700 µL sample (including any precipitate that may have formed) to the HiBind® RNA Mini Column.

13. Centrifuge at 10,000 x *g* for 1 minute.

14. Discard the filtrate and reuse the Collection Tube.

15. Repeat Steps 12-14 until all of the sample has been transferred to the column.

Optional: This the starting point of the optional on-membrane DNase I Digestion Protocol. Since the HiBind® matrix of the RNA Mini Column eliminates most DNA, DNase I digestion is not necessary for most downstream applications. However, certain sensitive RNA applications may require further DNA removal. If an additional RNA removal step is required, please continue to the DNase I Digestion Protocol found on Page 19. (See DNase I Digestion Set, E1091, for more information). If DNase I digestion is not required, proceed to Step 16.

16. Add 500 µL RNA Wash Buffer I to the HiBind® RNA Mini Column.

17. Centrifuge at 10,000 x *g* for 30 seconds.

18. Discard the filtrate and reuse the Collection Tube.

19. Add 500 µL RNA Wash Buffer II to the HiBind® RNA Mini Column.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 5 for instructions.

20. Centrifuge at 10,000 x *g* for 1 minute.

21. Discard the filtrate and reuse the Collection Tube.

22. Repeat Steps 19-21 for a second RNA Wash Buffer II wash step.

23. Centrifuge at maximum speed for 2 minutes to completely dry the HiBind® RNA Mini Column.

    Note: It is important to dry the HiBind® RNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

24. Transfer the HiBind® RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

25. Add 45-75 µL Nuclease-free Water.

    Note: Make sure to add water directly onto the HiBind® RNA Mini Column matrix.

26. Centrifuge at maximum speed for 2 minutes and store eluted RNA at -70°C.

    Note: Any combination of the following steps can be used to help increase RNA yield.

    - Heat the Nuclease-free Water to 70°C before adding to the column.
    - Increase the incubation time to 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Nuclease-free Water (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

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

Materials and Equipment to be Supplied by User:

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

Sample Disruption and Homogenization Equipment:

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

Before Starting:

- Prepare RNA Wash Buffer II according to the "Preparing Reagents" section on Page 5.
- Add 20 µL 2-mercaptoethanol per 1 mL RNA-Solv® Reagent.

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 Mini Column is 100 µg. The maximum amount of tissue that can be used with RNA-Solv® Reagent is 30 mg. Use the table on the following page as a guide 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)
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 the tissue and homogenize the tissue in 1 mL RNA-Solv® Reagent using one of the methods on Page 7. (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 they normally produce better yields.

3. Let sit at room temperature for 5 minutes.

4. Add 200 µL chloroform. Vortex for 20 seconds to mix thoroughly.

5. Let sit at room temperature for 2-3 minutes.

6. Centrifuge at maximum speed (≥12,000 x *g*) at 4°C for 15 minutes to separate the aqueous and organic phase.

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

7. Transfer the upper aqueous phase (~500 µL) into a new 1.5 mL microcentrifuge tube.

8. Add an equal volume of 70% ethanol. Vortex to mix thoroughly.

   Note: A precipitate may form at this point. This will not interfere with the RNA purification.

9. Insert a HiBind® RNA Mini Column into a 2 mL Collection Tube.

10. Transfer 700 µL sample (including any precipitate that may have formed) to the HiBind® RNA Mini Column.

11. Centrifuge at 10,000 x *g* for 1 minute.

12. Discard the filtrate and reuse the Collection Tube.

13. Repeat Steps 10-12 until all of the sample has been transferred to the column.

Optional: This the starting point of the optional on-membrane DNase I Digestion Protocol. Since the HiBind® matrix of the RNA Mini Column eliminates most DNA, DNase I digestion is not necessary for most downstream applications. However, certain sensitive RNA applications may require further DNA removal. If an additional RNA removal step is required, please continue to the DNase I Digestion Protocol found on Page 19. (See DNase I Digestion Set, E1091, for more information). If DNase I digestion is not required, proceed to Step 14.

14. Add 500 µL RNA Wash Buffer I to the HiBind® RNA Mini Column.

15. Centrifuge at 10,000 x *g* for 30 seconds.

16. Discard the filtrate and reuse the Collection Tube.

17. Add 500 µL RNA Wash Buffer II to the HiBind® RNA Mini Column.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 5 for instructions.

18. Centrifuge at 10,000 x *g* for 1 minute.

19. Discard the filtrate and reuse the Collection Tube.

20. Repeat Steps 17-19 for a second RNA Wash Buffer II wash step.

21. Centrifuge at maximum speed for 2 minutes to completely dry the HiBind® RNA Mini Column.

    Note: It is important to dry the HiBind® RNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

22. Transfer the HiBind® RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

23. Add 45-75 µL Nuclease-free Water.

    Note: Make sure to add water directly onto the HiBind® RNA Mini Column matrix.

24. Centrifuge at maximum speed for 2 minutes and store eluted RNA at -70°C.

    Note: Any combination of the following steps can be used to help increase RNA yield.

    - Heat the Nuclease-free Water to 70°C before adding to the column.
    - Increase the incubation time to 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Nuclease-free Water (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

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

Carry out lysis and 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

Before Starting:

- Assemble vacuum manifold according to the manufacturer's instructions.

After completing Steps 1-10 of the Animal Cell Protocol (Pages 8-10) or Steps 1-8 of the Animal Tissue Protocol (Pages 13-15), proceed with the following protocol.

1. Attach a HiBind® RNA Mini Column to the vacuum manifold.

2. Transfer the homogenized sample onto the HiBind® RNA Mini Column.

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

4. Turn off the vacuum.

5. Add 500 µL RNA Wash Buffer I to the HiBind® RNA Mini Column.

6. Switch on the vacuum source to draw the RNA Wash Buffer I through the column.

7. Turn off the vacuum.

8. Add 500 µL RNA Wash Buffer II to the HiBind® RNA Mini Column.

   Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 5 for instructions.

9. Switch on the vacuum source to draw the RNA Wash Buffer II through the column.

10. Turn off the vacuum.

11. Repeat Steps 8-10 for a second RNA Wash Buffer II wash step.

12. Insert a HiBind® RNA Mini Column into a 2 mL Collection Tube provided with this kit.

13. Centrifuge at maximum speed for 2 minutes to completely dry the HiBind® RNA Mini Column.

    Note: It is important to dry the HiBind® RNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

14. Transfer the HiBind® RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

15. Add 45-75 µL Nuclease-

Note: Make sure to add water directly onto the HiBind® RNA Mini Column matrix.
free Water.

16. Centrifuge at top speed for 2 minutes and store eluted RNA at -70°C.

    Note: Any combination of the following steps can be used to help increase RNA yield.

    - Heat the Nuclease-free Water to 70°C before adding to the column.
    - Increase the incubation time to 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Nuclease-free Water (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

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

Since the HiBind® matrix of the RNA Mini Column eliminates most DNA, DNase I digestion is not necessary for most downstream applications. However, certain sensitive RNA applications may require further DNA removal. (See DNase I Digestion Set, Cat# E1091 for further information).

After completing Steps 1-15 of the Animal Cell Protocol (Pages 8-10) or Steps 1-13 of the Animal Tissue Protocol (Pages 13-15), proceed with the following protocol.

All centrifugation steps used are performed at room temperature.

User Supplied Material:

- DNase I Digestion Set (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

Important Notes:

- DNase I is very sensitive and prone to physical denaturing. **Do not vortex the DNase I mixture.** Mix gently by inverting the tube.
- Freshly prepare DNase I stock solution right before RNA isolation.
- 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 and may reduce RNA yields and purity.
- All steps must be carried out at room temperature. Work quickly, but carefully.

2. Insert the HiBind® RNA Mini Column containing the sample into a 2 mL Collection Tube.

3. Add 250 µL RNA Wash Buffer I to the HiBind® RNA Mini Column.

4. Centrifuge at 10,000 x *g* for 1 minute.

5. Discard the filtrate and reuse the Collection Tube.

6. Add 75 µL DNase I digestion mixture directly onto the surface of the membrane of the HiBind® RNA Mini Column.

   Note: Pipet the DNase I directly onto the membrane. DNA digestion will not be complete if some of the mixture is retained on the wall of the HiBind® RNA Mini Column.

7. Let sit at room temperature for 15 minutes.

8. Add 250 µL RNA Wash Buffer I to the HiBind® RNA Mini Column.

9. Let sit at room temperature for 2 minutes.

10. Centrifuge at 10,000 x *g* for 1 minute.

11. Discard the filtrate and reuse the Collection Tube.

12. Add 500 µL RNA Wash Buffer II.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 8 for instructions.

13. Centrifuge at 10,000 x *g* for 1 minute.

14. Discard the filtrate and reuse the Collection Tube.

15. Repeat Steps 12-14 for a second RNA Wash Buffer II wash step.

16. Centrifuge at maximum speed for 2 minutes to completely dry the HiBind® RNA Mini Column matrix.

    Note: It is important to dry the HiBind® RNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

17. Place the column in a clean 1.5 mL microcentrifuge tube (not supplied).

18. Add 45-75 µL Nuclease-free Water.

    Note: Make sure to add water directly onto the HiBind® RNA Mini Column matrix.

19. Let sit at room temperature for 1 minute.

20. Centrifuge at maximum speed for 2 minutes and store eluted RNA at -70°C.

    Note: Any combination of the following steps can be used to help increase RNA yield.

    - Heat the Nuclease-free Water to 70°C before adding to the column.
    - Increase the incubation time to 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh Nuclease-free Water (this may increase the yield, but decrease the concentration).
    - Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

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	Nuclease-free Water is acidic and can dramatically lower Abs_{260} values. Use TE buffer to dilute RNA prior to spec trophotometric 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 I (100 mL)	PR030
RNA Wash Buffer II (100 mL)	PR031
DEPC Water (100 mL)	PR032
Homogenizer Mini Columns	HCR003
RNase-free DNase Set (5o preps)	E1091-01
RNase-free DNase Set (200 preps)	E1091-02
Proteinase K Solution	AC110/AC111