omega BIO-TEK
innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Total RNA Midi Kit

R6664-00	2 preps
R6664-02	25 preps

Manual Date: December 2018
Revision Number: v2.0

For Research Use Only

Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

omega-bio-tek
omegabiotek
omegabiotek

E.Z.N.A® Total RNA Midi Kit

Table of Contents

Manual Date: December 2018
Revision Number: v2.0

omega BIO-TEK
innovations in nucleic acid isolation

Introduction and Overview

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 midi-column spin technology, thereby permitting simultaneous processing of multiple samples. 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.® RNA purification system is ready for applications such as RT-PCR, Northern blotting, polyA+ RNA (mRNA) purification, nuclease protection, and *in vitro* translation.

The E.Z.N.A.® Total RNA Midi Kit can purify up to 600 µg total RNA from cultured eukaryotic cells, tissue, or bacteria. Normally, 5 x 10^6 - 1 x 10^8 eukaryotic cells, 5 x 10^8-1 x 10^{10} bacterial cells, or 25-200 mg tissue can be processed in a single experiment. Lysis of cells or tissue occurs under denaturing conditions. After the homogenization process, samples are applied to the HiBind® RNA Midi 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 eluted in Nuclease-free Water.

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 Midi Kit (Part# R6615) as it is specifically designed for effective hemolysis and hemoglobin removal, thereby giving higher RNA yields.

Each HiBind® RNA Midi Column can bind approximately 1 mg RNA. Using greater than 200 mg tissue or 1 x 10^8 cells is not recommended.

New in this Edition:

December 2018

- This manual has been edited for content and redesigned to enhance user readability.
- DEPC Water has been replaced with Nuclease-free Water. DEPC Water is no longer provided in this kit.
- PR032 (DEPC Water, 100 mL) has been discontinued and is no longer available to purchase.

Kit Contents

Product	R6664-00	R6664-02
Purifications	2	25
HiBind® RNA Midi Columns	2	25
15 mL Collection Tubes	2	25
TRK Lysis Buffer	10 mL	120 mL
RNA Wash Buffer I	10 mL	110 mL
RNA Wash Buffer II	5 mL	50 mL
Nuclease-free Water	2 mL	30 mL
User Manual	✓	✓

Storage and Stability

All E.Z.N.A.® Total RNA Midi Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature. Check buffers for precipitates before use. Redissolve any precipitates by warming to 37°C while gently shaking.

Important Notes

Please take a few minutes to read this booklet in its entirety to become familiar with the procedures. Prepare all materials required before starting to minimize RNA degradation.

- Whenever working with RNA, always wear gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.

- Equilibrate samples and reagents to room temperature before beginning this protocol. All steps should be carried out at room temperature unless otherwise noted. Work quickly, but carefully.

- Prepare all materials required before starting the procedure to minimize RNA degradation.

- Carefully apply the sample or solution to the center of the HiBind® RNA Midi Columns. Avoid touching the membrane with pipet tips.

- 2-mercaptoethanol is key in denaturing RNases and can be added to an aliquot of TRK Lysis Buffer before use. Add 20 µL 2-mercaptoethanol per 1 mL TRK Lysis Buffer. This mixture can be stored for 4 weeks at room temperature.

Quantification and Storage of RNA

To determine the concentration and purity of RNA, measure absorbance at 260 nm and 280 nm with a spectrophotometer. One OD unit measured at 260 nm corresponds to 40 µg/mL RNA. DEPC Water is slightly acidic and can dramatically lower absorbance values. We suggest that you dilute the sample in a buffered solution (TE) for spectrophotometric analysis. The A_{260}/A_{280} ratio of pure nucleic acids is 2.0, while an A_{260}/A_{280} ratio of 0.6 denotes pure protein. A ratio of 1.8-2.0 corresponds to 90%-100% pure nucleic acid. Phenol has a maximum absorbance at 270 nm and can interfere with spectrophotometric analysis of DNA or RNA. Store RNA samples at -70°C in water. Under these conditions, RNA is stable for more than a year.

Integrity of RNA

It is highly recommended that RNA quality be determined prior to beginning all downstream applications. The quality of RNA can be best assessed by denaturing agarose gel electrophoresis with ethidium bromide staining. The ribosomal RNA bands should appear as sharp, clear bands on the gel. The 28S band should appear to be double that of the 18S RNA band (23S and 16S if using bacteria). If the ribosomal RNA bands in any given lane are not sharp and appear to be smeared towards the smaller sized RNA, it is very likely that the RNA undergone degradation during the isolation, handling, or storage procedure. 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.

Preparing Reagents

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

Kit	100% Ethanol to be Added
R6664-00	20 mL
R6664-02	200 mL

2. Add 20 µL 2-mercaptoethanol per 1 mL TRK Lysis Buffer. This mixture can be stored for 4 weeks at room temperature.

Homogenization Techniques

Efficient sample disruption and homogenization is essential for successful total RNA isolation. Cell wall and plasma membrane disruption is necessary for the release of RNA from the sample and homogenization is necessary to reduce the viscosity of the lysates. Homogenization shears genomic DNA and other high-molecular-weight cell components creating a homogeneous lysate. Incomplete homogenization can cause the HiBind® RNA Midi Column to clog resulting in low or no yield.

Liquid Nitrogen Method

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

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

3. Grind tissue with a ceramic mortar and pestle under approximately 10 mL liquid nitrogen.

4. Pour the suspension into a pre-cooled 15 mL polypropylene tube.

   Note: Unless the tube is pre-cooled in liquid nitrogen, the suspension will boil vigorously and may cause loss of tissue.

5. Allow the liquid nitrogen to completely evaporate and add TRK Lysis Buffer.

6. Proceed to one of the homogenization steps below.

Homogenization - Choose one method below

1. **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.

2. **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 size of glass beads to use for RNA isolation are 0.5 mm for yeast/unicellular cells and 4-8 mm for animal tissue samples.

3. **Syringe Needle: Sample Disruption and Homogenization**
   High-molecular-weight DNA is responsible for the viscosity of cell lysates and can be shredded by passing the sample 10-20 times through a narrow gauge needle (19-21 G).

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

Materials and Equipment to be Supplied by User:

- Swing bucket centrifuge capable of at least 4,000 x *g*
- Vortexer
- RNase-free pipette tips
- RNase-free 15 mL centrifuge tubes
- 100% ethanol
- 70% ethanol
- 14.3M 2-mercaptoethanol (β-mercaptoethanol)
- Sample disruption and homogenization equipment
- Optional: PBS
- Optional: Trypsin
- Optional: RNase-free glass or polypropylene centrifuge tube

Before Starting:

- Prepare RNA Wash Buffer II and TRK Lysis Buffer according to the "Preparing Reagents" section on Page 6.

1. Determine the proper amount of starting material.

   Note: It is critical to use the correct number of starting cells in order to obtain optimal yield and purity. The maximum number of cells that can be processed is dependent on the specific RNA contents and type of cell line. The maximum binding capacity of the HiBind® RNA Midi Column is 1 mg. The maximum number of cells that TRK Lysis Buffer can efficiently lyse is 1 x 10^8. Use the following table as a guideline to select the correct starting material.

Average Yield of Total Cellular RNA

Source	Number of cells	RNA Yield
IC21	4 x 10^7	850 µg
Hela	7 x 10^7	1,000 µg
293HEK	6 x 10^7	850 µg
HIN3T3	7 x 10^7	1,000 µg

2. Harvest cells using one of the following methods. Do not use more than 1 x 10^8 cells.

   - For cells grown in suspension:

     1. Determine the number of cells.
     2. Centrifuge at 500 x *g* for 5 minutes.
     3. Aspirate and discard the supernatant.
     4. Proceed to Step 3 on Page 10.

   - For cells grown in a monolayer:

     Note: 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.

     - For direct cell lysis:

       1. Determine the number of cells.
       2. Aspirate and discard the cell culture medium.
       3. Immediately proceed to Step 3 on Page 10.

       Note: Incomplete removal of the cell-culture medium will inhibit lysis and dilute the lysate. This will affect the RNA binding conditions of the HiBind® RNA Midi Column and may reduce RNA yield.

     - To trypsinize and collect cells:

       1. Determine the cell number.
       2. Aspirate and discard the cell-culture medium and wash the cells with PBS. Incomplete removal of the cell-culture medium will inhibit trypsin. Multiple washes may be necessary for cells that are difficult to detach.
       3. Add 0.1-0.25% trypsin in a balanced salt solution.
       4. Incubate for 3-5 minutes to allow cells to detach. Check cells for detachment before proceeding to the next step.
       5. Add an equal volume of cell-culture medium containing serum to inactivate the trypsin.
       6. Transfer cells to an RNase-free glass or polypropylene centrifuge tube (not provided).
       7. Centrifuge at 500 x *g* for 5 minutes.
       8. Aspirate the supernatant.
       9. Proceed to Step 3 on Page 10.

          Note: Incomplete removal of the cell-culture medium 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 with TRK Lysis Buffer. Do not use more than 1 x 10^8 cells.

   Note: Add 20 µL β-ME per 1 mL TRK Lysis Buffer before use.

   - 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.
   - For direct lysis of cells grown in a monolayer, add the appropriate amount of TRK Lysis Buffer directly to the dish based on the table below.

Number of Cells	Amount of TRK Lysis Buffer
$5x10^6$ - $5x10^7$	2 mL
$5x10^7$ - $1x10^8$	4 mL

4. Homogenize cells with a rotor-stator homogenizer until the sample is uniformly homogenized. Alternatively, sample can be homogenized by using the syringe and needle method as described on Page 7.

   Note: Incomplete homogenization of the sample will cause lower yields and clogging of the column. It is recommended to homogenize the sample with a rotor-stator homogenizer since it can produces better yields.

5. Add an equal volume 70% ethanol (2 mL or 4 mL). Vortex to mix thoroughly. ***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. Transfer the sample (including any precipitate that may have formed) to a HiBind® RNA Midi Column preinserted into a 15 mL Collection Tube (provided).

7. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

8. Repeat Steps 6-7 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 Midi 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 24. (See DNase I Digestion Set, (E1091) for more information). If DNase I digestion is not required, proceed to Step 9.

9. Add 3.5 mL RNA Wash Buffer I.

10. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

11. Add 3 mL RNA Wash Buffer II.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 6 for instructions.

12. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

13. Add 3.5 mL RNA Wash Buffer II.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 6 for instructions.

14. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

15. Centrifuge the empty HiBind® RNA Midi Column for 10 minutes at 4,000 x *g* to completely dry the HiBind® matrix.

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

16. Transfer the HiBind® RNA Midi Column to a clean 15 mL centrifuge tube (not provided).

17. Add 250-500 µL Nuclease-free Water. Make sure to add the water directly onto the HiBind® RNA Midi Column matrix.

18. Centrifuge at 4,000 x *g* for 5 minutes and store RNA at -70°C. A second elution may be necessary if the expected yield of RNA is >500 µg.

    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 Midi Kit - Animal Tisse Protocol

Materials and Equipment to be Supplied by User:

- Swing bucket centrifuge capable of at least 4,000 x *g*
- Vortexer
- RNase-free pipette tips
- RNase-free 15 mL centrifuge tubes
- 100% ethanol
- 70% ethanol
- 14.3M 2-mercaptoethanol (β-mercaptoethanol)
- Sample disruption and homogenization equipment

Before Starting:

- Prepare RNA Wash Buffer II and TRK Lysis Buffer according to the "Preparing Reagents" section on Page 6.

1. Determine the proper amount of starting material.

   Note: It is critical to use the correct amount of starting tissue in order to obtain optimal yield and purity. The maximum amount of tissue that can be processed is dependent on the specific RNA contents and type of tissue. The maximum binding capacity of the HiBind® RNA Midi Column is 1 mg. The maximum amount of tissue that TRK Lysis Buffer can efficiently lyse is 250 mg. Use the table below as a guideline to select the correct starting material. If you have no information regarding your starting material, use 100 mg as a starting amount. Given the yield and quality of RNA obtained from 100 mg, adjust the starting amount in the next purification.

Average Yield of Total Cellular RNA from Mouse Tissue

Source	Amount of Tissue	RNA Yield
Brain	100	100 µg
Kidney	150	450 µg
Liver	150	650 µg
Heart	200	100 µg
Spleen	150	500 µg
Lung	100	120 µg
Pancreas	100	400 µg
Thymus	100	200 µg

2. Disrupt tissue with TRK Lysis Buffer. Do not use more than 250 mg tissue.

   Note: Add 20 µL β-ME per 1 mL TRK Lysis Buffer before use. Add the appropriate amount of TRK Lysis Buffer based on the table below.

Amount of Tissue	Amount of TRK Lysis Buffer
20-120 mg	2 mL
120-250 mg	4 mL

3. Homogenize the tissue with a rotor-stator homogenizer until the sample is uniformly homogenized. Alternatively, the sample can be homogenized by using the syringe and needle method as described on Page 7.

   Note: Incomplete homogenization of the sample will cause lower yields and clogging of the column. It is recommended to homogenize the sample with a rotor-stator homogenizer since it can produces better yields.

4. Centrifuge at 4,000 x *g* for 10 minutes.

5. Carefully transfer the cleared lysate to a clean 15 mL centrifuge tube (not provided).

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

6. Add an equal volume 70% ethanol (2 mL or 4 mL). Vortex to mix thoroughly. ***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.

7. Transfer the sample (including any precipitate that may have formed) to a HiBind® RNA Midi Column preinserted into a 15 mL Collection Tube (provided).

8. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

9. Repeat Steps 7-8 until all the sample has been transferred to the HiBind® RNA Midi Column.

Optional: This the starting point of the optional on-membrane DNase I Digestion Protocol. Since the HiBind® matrix of the RNA Midi 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 24. (See DNase I Digestion Set, (E1091) for more information). If DNase I digestion is not required, proceed to Step 10.

10. Add 3 mL RNA Wash Buffer I.

11. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

12. Add 3 mL RNA Wash Buffer II.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 6 for instructions.

13. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

14. Add 3.5 mL RNA Wash Buffer II.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 6 for instructions.

15. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

16. Centrifuge the empty HiBind® RNA Midi Column for 10 minutes at 4,000 x *g* to completely dry the HiBind® matrix.

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

17. Transfer the HiBind® RNA Midi Column to a clean 15 mL centrifuge tube (not provided).

18. Add 250-500 µL Nuclease-free Water. Make sure to add the water directly onto the HiBind® RNA Midi Column matrix.

19. Centrifuge at 4,000 x *g* for 5 minutes and store RNA at -70°C. A second elution may be necessary if the expected yield of RNA is >500 µg.

    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 Midi Kit - Heart, Muscle, and Skin Tissue Protocol

Due to the rich content of contractile proteins, connective tissue, and collagen, it can be difficult to isolate RNA from heart, muscle, and skin tissue using the standard E.Z.N.A.® Total RNA Midi Kit Animal Tissue Protocol. The following protocol is a modified version that has added a Proteinase K digestion step to improve tissue lysis. Proteinase K is not included and must be purchased separately. Please refer to the "Ordering Information" section on Page 28.

Materials and Equipment to be Supplied by User:

- Swing bucket centrifuge capable of at least 4,000 x *g*
- Water bath, oven, or heat block capable of 55°C
- Vortexer
- RNase-free pipette tips
- RNase-free 15 mL centrifuge tubes
- 100% ethanol
- ddH_2O
- Proteinase K Solution (Part# AC116, 20 mg/mL)
- 14.3M 2-mercaptoethanol (β-mercaptoethanol)
- Sample disruption and homogenization equipment

Before Starting:

- Prepare RNA Wash Buffer II and TRK Lysis Buffer according to the "Preparing Reagents" section on Page 6.
- Heat water bath, oven, or heat block to 55°C.

1. Determine the proper amount of starting material.

   Note: It is critical to use the correct amount of starting tissue in order to obtain optimal yield and purity. The maximum amount of tissue that can be processed is dependent on the specific RNA contents and type of tissue. The maximum binding capacity of the HiBind® RNA Midi Column is 1 mg. The maximum amount of tissue that TRK Lysis Buffer can efficiently lyse is 250 mg. If you have no information regarding your starting material, use 100 mg as a starting amount. Given the yield and quality of RNA obtained from 100 mg, adjust the starting amount in the next purification.

2. Disrupt tissue in 2 mL TRK Lysis Buffer. Do not use more than 250 mg tissue.

   Note: Add 20 µL β-ME per 1 mL TRK Lysis Buffer before use.

3. Homogenize the tissue with a rotor-stator homogenizer until the sample is uniformly homogenized. Alternatively, the sample can be homogenized by using the syringe and needle method as described on Page 7.

   Note: Incomplete homogenization of the sample will cause lower yields and clogging of the column. It is recommended to homogenize the sample with a rotor-stator homogenizer since it can produces better yields.

4. Add 4 mL ddH_2O and 250 µL Proteinase K Solution. Pipet up and down to mix thoroughly.

5. Incubate at 55°C for 25 minutes.

6. Centrifuge at 4,000 x *g* for 10 minutes.

7. Carefully transfer the cleared lysate to a clean 15 mL centrifuge tube (not provided).

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

8. Add 0.5 volumes 100% ethanol. Vortex to mix thoroughly. ***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.

9. Transfer the sample (including any precipitate that may have formed) to a HiBind® RNA Midi Column preinserted into a 15 mL Collection Tube (provided).

10. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

11. Repeat Steps 9-10 until all the sample has been transferred to the HiBind® RNA Midi Column.

Optional: This the starting point of the optional on-membrane DNase I Digestion Protocol. Since the HiBind® matrix of the RNA Midi 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 24. (See DNase I Digestion Set, (E1091) for more information). If DNase I digestion is not required, proceed to Step 12.

12. Add 3 mL RNA Wash Buffer I.

13. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

14. Add 3 mL RNA Wash Buffer II.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 6 for instructions.

15. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

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

17. Centrifuge the empty HiBind® RNA Midi Column for 10 minutes at 4,000 x *g* to completely dry the HiBind® matrix.

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

18. Transfer the HiBind® RNA Midi Column to a clean 15 mL centrifuge tube (not provided).

19. Add 250-500 µL Nuclease-free Water. Make sure to add the water directly onto the HiBind® RNA Midi Column matrix.

20. Centrifuge at 4,000 x *g* for 5 minutes and store RNA at -70°C. A second elution may be necessary if the expected yield of RNA is >400 µg.

    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 Midi Kit - Bacterial Protocol

The E.Z.N.A.® Total RNA Kit can be modified for the isolation of RNA from bacterial cultures. Only cells growing at log phase should be used. Measured at 600 nm, an OD of 0.5-1.0 corresponds to ~1 x 10^{10} cells per mL. This method is suitable for no more than 1 x 10^{10} cells.

Materials and Equipment to be Supplied by User:

- Swing bucket centrifuge capable of at least 4,000 x *g*
- Vortexer
- RNase-free pipette tips
- RNase-free 15 mL centrifuge tubes
- 100% ethanol
- TE Buffer
- Lysozyme
- 14.3M 2-mercaptoethanol (β-mercaptoethanol)

Before Starting:

- Prepare RNA Wash Buffer II and TRK Lysis Buffer according to the "Preparing Reagents" section on Page 6.
- Prepare TE Buffer and lysozyme as follows:
  - For gram-negative bacteria, add 1 mg lysozyme per 1 mL TE Buffer.
  - For gram-positive bacteria, add 4 mg lysozyme per 1 mL TE Buffer.

1. Centrifuge 5 x 10^9 cells at 4,000 x *g* for 5 minutes. Discard the supernatant and add 500 µL TE Buffer containing lysozyme. Resuspend the cells completely and let sit at room temperature for 7-10 minutes.

2. Add 2 mL TRK Lysis Buffer. Pipet up and down to mix.

   Note: Add 20 µL β-ME per 1 mL TRK Lysis Buffer before use.

3. Centrifuge at 4,000 x *g* for 10 minutes.

4. Transfer the cleared supernatant to a new 15 mL centrifuge tube (not provided).

5. Add 1.4 mL 100% ethanol. Vortex to mix thoroughly.

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

6. Transfer the sample (including any precipitate that may have formed) to a HiBind® RNA Midi Column preinserted into a 15 mL Collection Tube (provided).

7. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

Optional: This the starting point of the optional on-membrane DNase I Digestion Protocol. Since the HiBind® matrix of the RNA Midi 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 24. (See DNase I Digestion Set, (E1091) for more information). If DNase I digestion is not required, proceed to Step 8.

8. Add 3 mL RNA Wash Buffer I.

9. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

10. Add 3 mL RNA Wash Buffer II.

    Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 6 for instructions.

11. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

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

13. Centrifuge the empty HiBind® RNA Midi Column for 10 minutes at 4,000 x *g* to completely dry the HiBind® matrix.

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

14. Transfer the HiBind® RNA Midi Column to a clean 15 mL centrifuge tube (not provided).

15. Add 250-500 µL Nuclease-free Water. Make sure to add the water directly onto the HiBind® RNA Midi Column matrix.

16. Centrifuge at 4,000 x *g* for 5 minutes and store RNA at -70°C. A second elution may be necessary if the expected yield of RNA is >500 µg.

    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 Midi Kit - DNase I Digestion Protocol

Since the HiBind® matrix of the RNA Midi 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 1-8 of the Animal Cell Protocol (Pages 8-10), Steps 1-9 of the Animal Tissue Protocol (Pages 13-15), Steps 1-11 of the Heart, Muscle, and Skin Protocol (Pages 17-19), or Steps 1-7 of the Bacterial Protocol (Pages 21-22), proceed with the following protocol.

User Supplied Material:

- DNase I Digestion Set (E1091)

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

Buffer	Volume per Prep
E.Z.N.A.® DNase I Digestion Buffer	176 µL
RNase-free DNase I (20 Kunitz/µL)	4 µL
Total Volume	180 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. Add 1.5 mL RNA Wash Buffer I

3. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

4. Add 180 µL DNase I digestion mixture directly onto the surface of the the HiBind® RNA Midi Column matrix.

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

5. Let sit at room temperature for 15 minutes.

6. Add 1.5 mL RNA Wash Buffer I to the HiBind® RNA Midi Column.

7. Let sit at room temperature for 5 minutes.

8. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

9. Add 3 mL RNA Wash Buffer II.

   Note: RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 6 for instructions.

10. Centrifuge at 4,000 x *g* for 5 minutes. Discard the filtrate and reuse the collection tube.

11. Add 3.5 mL RNA Wash Buffer II.

12. Centrifuge at 4,000 x *g* for 5 minutes.

13. Centrifuge the empty column at 4,000 x *g* for 10 minutes to completely dry the HiBind® RNA Midi Column matrix.

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

14. Transfer the HiBind® RNA Midi Column to a new 15 mL centrifuge tube (not provided).

15. Add 250-500 µL Nuclease-free Water.

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

16. Centrifuge at 4,000 x *g* for 5 minutes and store 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 **1-800-832-8896**.

Problem	Cause	Solution
Little or no RNA eluted	RNA remains on the column	• Repeat elution. • Preheat Nuclease-free Water to 70°C prior to elution. • Incubate column for 10 minutes prior to centrifugation.
	Column is overloaded	Reduce the quantity of starting material.
Problem	Cause	Solution
Clogged column	Incomplete homogenization	• Completely homogenize the sample. • Increase centrifugation time. • Reduce the amount of starting material
Problem	Cause	Solution
Degraded RNA	Source	• 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 that RNA Wash Buffer II has been diluted with 100% ethanol before use. • RNA Wash Buffer II must be stored and used at room temperature. • Repeat wash with RNA Wash Buffer II.
Problem	Cause	Solution
DNA contamination		Digest with RNase-free DNase and inactivate at 75°C for 5 minutes.

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, 25 mL	PR031
Nuclease-free Water, 1000 mL	PD092
RNase-free DNase I Set, 50 preps	E1091
Proteinase K Solution, 10 mL	AC116

For more purification solutions, visit **www.omegabiotek.com**

AVAILABLE FORMATS


Spin Columns

96-Well Silica Plates


Mag Beads

SAMPLE TYPES


Blood / Plasma

Plasmid


Cultured Cells


Plant & Soil


NGS Clean Up


Tissue


FFPE


Fecal Matter

omega BIO-TEK

innovations in nucleic acid isolation

Omega Bio-tek, Inc.
400 Pinnacle Way, Suite 450
Norcross, GA 30071
www.omegabiotek.com

770-931-8400
770-931-0230
info@omegabiotek.com

omega-bio-tek
omegabiotek
omegabiotek