# E.Z.N.A.® Bacterial RNA Kit

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omega BIO-TEK
innovations in nucleic acid isolation

# Introduction and Overview

E.Z.N.A.® Bacterial RNA Kit allows rapid and reliable isolation of high-quality total cellular RNA from a wide variety of bacterial species. Up to 1 x $10^9$ bacterial cells can be processed. The system combines the reversible nucleic acid-binding properties of Omega Bio-Tek's HiBind® matrix with the speed and versatility of spin column technology to yield approximately 50-100 µg RNA. There are no organic extractions, thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel. Purified RNA has $A_{260}/A_{280}$ ratios of 1.8-2.0 and is suitable for the following applications:

- RT-PCR
- Northern Analysis
- Differential display
- Poly A+ RNA selection

If using the E.Z.N.A.® Bacterial RNA Kit for the first time, please read this booklet to become familiar with the procedures. Bacterial cells are grown to log-phase and harvested. Bacterial cell walls are removed by lysozyme digestion. Following lysis, binding conditions are adjusted and the sample is applied to HiBind® RNA Mini Column. Two rapid wash steps remove trace salt and protein contaminants and RNA is eluted in water or low ionic strength buffer. Purified RNA can be directly used in downstream applications without the need for further purification.

**New in this Edition:**

November 2018:
- 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.

July 2017:
- This manual has been edited for content and redesigned to enhance user readability.

# Kit Contents

| Product | R6950-00 | R6950-01 | R6950-02 |
|---|---|---|---|
| Purifications | 5 | 50 | 200 |
| HiBind® RNA Mini Columns | 5 | 50 | 200 |
| 2 mL Collection Tubes | 15 | 150 | 600 |
| BRK Lysis Buffer | 2 mL | 20 mL | 80 mL |
| RNA Wash Buffer I | 5 mL | 50 mL | 180 mL |
| RNA Wash Buffer II | 5 mL | 12 mL | 50 mL |
| Nuclease-free Water | 2 mL | 10 mL | 30 mL |
| Lysozyme | 8 mg | 80 mg | 4 x 80 mg |
| Glass Powder | 200 mg | 2 g | 8 g |
| User Manual | ✓ | ✓ | ✓ |

# Storage and Stability

All of the E.Z.N.A.® Bacterial RNA Kit components are guaranteed for at least 24 months from the date of purchase when stored as follows. Lysozyme should be stored at -20°C. All remaining components should be stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in some buffers. Dissolve such deposits by warming the solution at 37°C and 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 Mini Columns. Avoid touching the membrane with pipet tips.

## 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. Nuclease-free water is slightly acidic and can lower $A_{260}/A_{280}$ ratios. Use TE buffer to dilute RNA prior to 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.

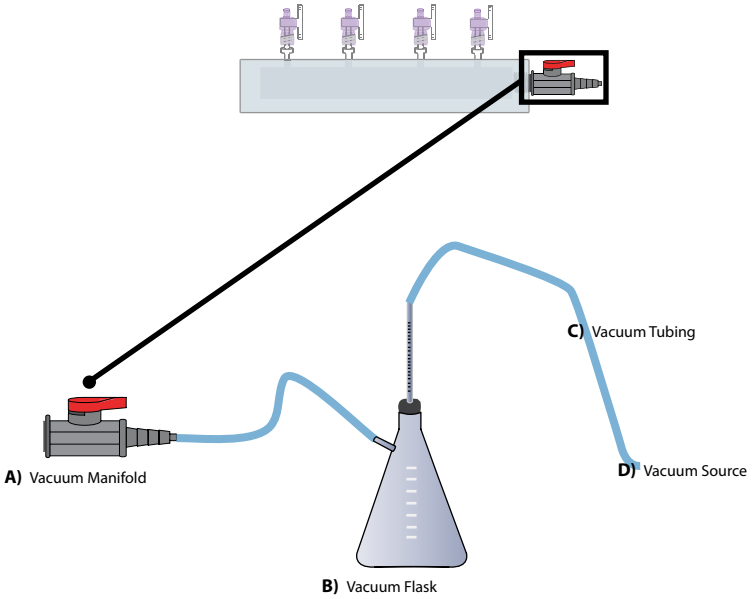
# Guideline for Vacuum Manifold

**The following is required for use with the Vacuum Protocol:**

**A)** Vacuum Manifold
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)

| Conversion from millibars: | Multiply by: |
|---|---|
| millimeters of mercury (mmHg) | 0.75 |
| kilopascals (kPa) | 0.1 |
| inches of mercury (inHg) | 0.0295 |
| Torrs (Torr) | 0.75 |
| atmospheres (atm) | 0.000987 |
| pounds per square inch (psi) | 0.0145 |

**Illustrated Vacuum Setup:**



# Preparing Reagents

1. Prepare a lysozyme stock solution (15 mg/mL) as follows and store aliquots at -20°C.

| Kit | TE Buffer to be Added |
|---|---|
| **R6950-00** | 530 µL |
| **R6950-01** | 5.3 mL |
| **R6950-02** | 5.3 mL to each bottle |

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

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

| Kit | 100% Ethanol to be Added |
|---|---|
| **R6950-00** | 20 mL |
| **R6950-01** | 48 mL |
| **R6950-02** | 200mL |

## E.Z.N.A.® Bacterial RNA Kit - Centrifugation Protocol

This method allows bacterial RNA isolation from up to 3 mL LB culture.

**Materials and Equipment to be Supplied by User:**

- Refrigerated centrifuge capable of 5,000 x *g*
- Microcentrifuge capable of 13,000 x *g*
- Shaking heat block, water bath, or incubator capable of 30°C
- Heat block, water bath, or incubator capable of 70°C
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 2-mercaptoethanol
- 100% ethanol
- TE Buffer

**Before Starting:**

- Prepare lysozyme, BRK Lysis Buffer, and RNA Wash Buffer II according to the "Preparing Reagents" section on Page 7.
- Set refrigerated centrifuge to 4°C.
- Set shaking heat block, water bath, or incubator to 30°C.
- Set heat block, water bath, or incubator to 70°C.

1. Culture bacteria in LB media to log phase. Do not use an overnight culture.

2. Centrifuge no more than 3 mL culture (<5 x $10^8$ bacteria) at 4,000 x *g* for 10 minutes at 4°C.

3. Aspirate and discard the media.

4. Add 100 µL lysozyme. Vortex at maximum speed for 30 seconds.

   **Note:** Lysozyme must be resuspended with TE Buffer before use. Please see Page 7 for instructions.

   **Note:** The amount of enzyme required and/or the incubation time may need to be modified depending on the bacterial strain used. Complete digestion of the cell wall is essential for efficient lysis. For some bacteria, other enzymes may be more effective.

5. Incubate at 30°C for 10 minutes with shaking. If a shaking water bath or incubator isnot available, incubate the samples and shake or briefly vortex every 2 minutes.

6. Add 350 µL BRK Lysis Buffer and 25 mg Glass Powder. Vortex at maximum speed for 5 minutes.

   **Note:** Add 20 µL 2-mercaptoethanol per 1 mL BRK Lysis Buffer before use. Please see Page 7 for instructions.

7. Centrifuge at maximum speed (≥13,000 x *g*) for 5 minutes.

8. Transfer 400 µL supernatant to a 1.5 mL microcentrifuge tube (not provided).

9. Incubate at 70°C for 5 minutes.

10. Centrifuge at maximum speed for 2 minutes.

11. Transfer the supernatant to a new 1.5 mL microcentrifuge tube.

12. Add 280 µL 100% ethanol. Vortex at maximum speed for 15 seconds.

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

14. Transfer the entire sample to the HiBind® RNA Mini Column, including any precipitate that may have formed.

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

16. 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 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 15. See RNase-free DNase I Set (Cat#E1091) for more information. If DNase I digestion is not required, proceed to Step 17.

17. Add 400 µL RNA Wash Buffer I.

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

19. Discard the filtrate and the collection tube.

20. Insert the HiBind® RNA Mini Column into a new 2 mL Collection Tube.

21. Add 500 µL RNA Wash Buffer II.

    **Note:** RNA Wash Buffer II must be diluted with 100% ethanol before use. Please see Page 7 for instructions.

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

23. Discard the filtrate and reuse the collection tube.

24. Repeat Steps 21-23 for a second RNA Wash Buffer II wash step.

25. Centrifuge the empty HiBind® DNA Mini Column at maximum speed for 2 minutes to dry the column.

    **Note:** This step is critical for removal of trace ethanol that may interfere with downstream applications.

26. Transfer the HiBind® RNA Mini Column into a new 1.5 mL microcentrifuge tube (not provided).

27. Add 50-100 µL Nuclease-free Water.

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

28. Let sit at room temperature for 2 minutes.

29. Centrifuge at 10,000 x *g* for 1 minute and store eluted RNA at -70°C.

    **Note:** If the expected RNA yield is more than 60 µg, repeat Steps 27-29 for a second elution step.

    **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.® Bacterial RNA Kit - Vacuum Protocol

**Note:** Please read through previous sections of this manual before proceeding with this protocol. Steps 1-12 from the Bacterial RNA Centrifugation Protocol should be completed before loading the sample to the HiBind® RNA Mini Column. Instead of continuing with centrifugation, follow the steps below.

**Materials and Equipment to be Supplied by User:**

- Vacuum manifold
- Vacuum source
- Refrigerated centrifuge capable of 5,000 x *g*
- Microcentrifuge capable of 13,000 x *g*
- Shaking heat block, water bath, or incubator capable of 30°C
- Heat block, water bath, or incubator capable of 70°C
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 2-mercaptoethanol
- 100% ethanol
- TE Buffer

**Before Starting:**

- Prepare lysozyme, BRK Lysis Buffer, and RNA Wash Buffer II according to the "Preparing Reagents" section on Page 7.
- Set refrigerated centrifuge to 4°C.
- Set shaking heat block, water bath, or incubator to 30°C.
- Set heat block, water bath, or incubator to 70°C.

1. Prepare the vacuum manifold according to manufacturer's instructions.

2. Connect the HiBind® RNA Mini Column to the vacuum manifold.

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

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

5. Turn off the vacuum.

6. Add 500 µL RNA Wash Buffer I.

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

8. Turn off the vacuum.

9. 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.

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

11. Turn off the vacuum.

12. Repeat Steps 9-11 for a second RNA Wash Buffer II wash step.

13. Transfer the HiBind® RNA Mini Column to a 2 mL Collection Tube.

14. Centrifuge at maximum speed (≥13,000 x *g*) 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.

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

16. Add 50-100 µL Nuclease-free Water.

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

17. Let sit at room temperature for 2 minutes.

18. Centrifuge at maximum speed for 1 minute 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 I - 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. Please see RNase-free DNase I Set (Cat#E1091) for further information.

After completing Steps 1-16 of the Centrifugation Protocol (Pages 8-9), proceed with the following protocol.

**Additional User Supplied Materials:**

- RNase-free DNase I Set (Cat#E1091)

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

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

**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. Transfer the HiBind® RNA Mini Column containing the sample from Step 16 (Page 9) into a new 2 mL Collection Tube.

3. Add 200 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 200 µL RNA Wash Buffer I to the HiBind® RNA Mini Column.

9. Let sit at room temperature for 5 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. Transfer the column to a new 1.5 mL microcentrifuge tube (not provided).

18. Add 50-100 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 2 minutes.

20. Centrifuge at 10,000 x *g* for 1 minute 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 **1-800-832-8896**.

| Problem | Cause | Solution |
|---|---|---|
| Little or no RNA eluted | RNA remains on the column | • Repeat elution.<br>• Preheat Nuclease-free Water to 70°C prior to elution.<br>• Let sit at room temperature for 10 minutes with Nuclease-free Water prior to centrifugation. |
| | Column is overloaded | Reduce the amount of starting material. |
| | Bacterial cell wall is not completely removed | Use longer incubation time for lysozyme digestion or add more lysozyme. |
| Problem | Cause | Solution |
| Clogged column | Incomplete disruption or lysis of bacterial. | • Use longer incubation time for lysozyme.<br>• Increase centrifugation time.<br>• Reduce amount of starting material. |
| Degraded RNA | Source | • Follow protocol closely, and work quickly.<br>• Make sure that 2-mercaptoethanol is added to BRK Lysis Buffer. |
| | RNase contamination | • Ensure not to introduce RNase during the procedure.<br>• Check buffers for RNase contamination. |
| Problem | Cause | Solution |
| Problem in downstream applications | Salt carry-over during elution | • RNA Wash Buffer II must be diluted with 100% ethanol as indicated on bottle.<br>• Store RNA Wash Buffer II at room temperature.<br>• Repeat RNA Wash Buffer II wash step. |
| DNA contamination | Co-purification of DNA | Digest with RNase-free DNase I and inactivate at 75°C for 5 minutes. |
| Low Abs ratios | RNA diluted in acidic buffer or water | Nuclease-free Water is acidic and can dramatically lower $A_{260}$ values. Use TE buffer (pH 8) to dilute RNA prior to analysis. |

# Ordering Information

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

**Notes:**