omega BIO-TEK
innovations in nucleic acid isolation

# Product Manual

## E.Z.N.A.® Fungal RNA Mini Kit

| | |
|---|---|
| R6840-00 | 5 preps |
| R6840-01 | 50 preps |

**Manual Date: April 2023**
**Revision Number: v4.1**

**For Research Use Only**


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# E.Z.N.A.® Fungal RNA Mini Kit

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# Introduction

E.Z.N.A.® Fungal RNA Mini Kit provides a rapid and reliable method for isolation of total RNA from a wide variety of fungal samples. This kit does not require the use of cumbersome or expensive shredding/homogenizing accessories as an attempt to shear viscous fungal lysates. Rather, this method involves a simple and rapid precipitation step for removal of much of the polysachrides and phenolic compounds commonly found in fungal tissues. In combination with HiBind® RNA Mini Column, this method permits purification of high-quality RNA from as much as 200 mg tissue. The system is efficient enough to allow total RNA from as little as 10 mg tissue or 100 cells. Typical yields are shown below in Table 1. The procedure involves no organic extractions, thus reducing plastic waste and hands-on time. E.Z.N.A.® Fungal RNA Mini Kits are ideal for processing multiple fungal samples in parallel in 1 hour. 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

**Table 1. Yields obtained with E.Z.N.A.® Fungal RNA Mini Kit**

| | |
|---|---|
| *Acremonium chrysogenum* | 50 µg |
| *Fusarium avenaceum* | 37 µg |
| Mushrooms | 43 µg |

**New in this Edition:**

April 2023:
- Units have been added for reagent preparation.

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.

## 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.

# Kit Contents

| Product | R6840-00 | R6840-01 |
|---|---|---|
| Purifications | 5 | 50 |
| HiBind® RNA Mini Columns | 5 | 50 |
| Homogenizer Mini Columns | 5 | 50 |
| 2 mL Collection Tubes | 15 | 150 |
| NTL Lysis Buffer | 5 mL | 40 mL |
| SP Buffer | 2 mL | 10 mL |
| RB Buffer* | 5 mL | 30 mL |
| RNA Wash Buffer I | 5 mL | 30 mL |
| RNA Wash Buffer II | 5 mL | 12 mL |
| Nuclease-free Water | 2 mL | 30 mL |
| User Manual | ✓ | ✓ |

* RB Buffer and NTL Lysis Buffer contain a chaotropic salt. Use gloves and protective eyewear when handling these solutions.

## Storage and Stability

All of the E.Z.N.A.® Fungal RNA Mini Kit components are guaranteed for at least 12 months from the date of purchase when 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.

# Preparing Reagents

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

| Kit | 100% Ethanol to be Added |
|---|---|
| **R6840-00** | 20 mL |
| **R6840-01** | 48 mL |

- Add 20 µL 2-mercaptoethanol (β-mercaptoethanol) per 1 mL RB Buffer and/or NTL Lysis Buffer depending on the protocol. This mixture can be stored for 4 weeks at room temperature.

# 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.

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

## Standard Protocol

This protocol is suitable for most fresh or frozen tissue samples allowing for efficient recovery of RNA. However, due to the tremendous variation in water and polysaccharide content of fungal samples, sample size should be limited to ≤100 mg. The method isolates sufficient RNA for a few tracks on a standard Northern assay. Collect tissue in a 1.5 mL or 2.0 mL microcentrifuge tube and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable homogenization pestles. Alternatively, one can allow liquid nitrogen to evaporate and then store samples at -70°C for later use. Do not allow the samples to thaw. Use disposable pestles only once. Alternatively, a small clean mortar and pestle can be used. The above methods for disrupting fungal tissue cannot be replaced with mechanical homogenizers.

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

- Microcentrifuge capable of 13,000*g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Incubator, heat block, or water bath capable of 65°C
- 2-mercaptoethanol
- 100% ethanol
- Liquid nitrogen for freezing/disrupting samples
- Optional: DNase I Digestion Set (E1091)

**Before Starting:**

- Prepare RNA Wash Buffer II and RB Buffer according to the "Preparing Reagents" section on Page 5
- Heat Nuclease-free Water to 65°C

**Notes:** Use extreme caution when handling liquid nitrogen.
All centrifugation steps must be carried out at room temperature.

1. Transfer up to 100 mg frozen ground fungal tissue to a 1.5 mL or 2 mL microcentrifuge tube (not provided).

   **Note:** We recommend starting with 50 mg of tissue at first. If results obtained are satisfactory then increase the amount of starting material.

2. Immediately add 500 µL RB Buffer. Samples should not be allowed to thaw before RB Buffer is added.

   **Note:** RB Buffer must be mixed with 2-mercaptoethanol before use. Please see Page 5 for instructions.

3. Vortex vigorously to make sure that all of the clumps have dispersed. RNA cannot be effectively extracted from a clumped sample.

4. Insert a Homogenizer Mini Column into a 2 mL Collection Tube.

5. Transfer the lysate to the Homogenizer Mini Column.

6. Centrifuge at 13,000*g* for 5 minutes at room temperature.

7. Carefully transfer the supernatant of the filtrate to a new 1.5 mL or 2 mL microcentrifuge tube. Do not disturb the pellet or transfer any debris.

   **Tip:** In most cases 450 µL supernatant can easily be removed. This will require 225 µL 100% ethanol in the next step. Depending on the sample, the volume of supernatant may vary. After transferring to a new tube, measure the volume and add the correct amount of ethanol in the next step.

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

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

10. Transfer the entire sample from Step 8, including any precipitates that may have formed, to the HiBind® RNA Mini column.

11. Centrifuge at 10,000*g* for 30 seconds at room temperature.

12. 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 DNA removal step is required, please continue to the DNase I Digestion Protocol found on Page 15. (See DNase I Digestion Set, (E1091) for more information). If DNase I digestion is not required, proceed to Step 13.

13. Add 500 µL RNA Wash Buffer I.

14. Centrifuge at 10,000*g* for 30 seconds.

15. Discard the filtrate and the collection tube.

16. Transfer the HiBind® RNA Mini Column into a new 2 mL Collection Tube.

17. Add 500 µL RNA Wash Buffer II.

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

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

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 or 2 mL microcentrifuge tube (not provided).

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

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

24. Centrifuge at top speed for 1 minute and store eluted RNA at -70°C.

    **Note:** A second elution into the same tube may be necessary if the expected yield of RNA is >50 µg. Alternatively, RNA may be eluted with a greater volume of Nuclease-free Water. While an additional elution step will increase total RNA yield, the concentration will be lowered since more than 80% of RNA is recovered with the first elution.

## Protocol for Difficult Sample Types

Certain fungal samples are very difficult for RNA isolation because of the amount of material and type of secondary metabolites. This method involves a simple and rapid precipitation step for removal of much of the polysaccharides and phenolic compounds commonly found in fungal tissues. Use this protocol when standard protocol did not yield RNA or provided a lower yield.

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

- Microcentrifuge capable of 10,000*g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Incubator, heat block, or water bath capable of 65°C
- 2-mercaptoethanol
- 100% ethanol
- 70% ethanol
- Isopropanol
- Liquid nitrogen for freezing/disrupting samples
- Optional: DNase I Digestion Set (E1091)

**Before Starting:**

- Prepare RNA Wash Buffer II and NTL Lysis Buffer according to the "Preparing Reagents" section on Page 5
- Heat Nuclease-free Water to 65°C

**Notes:** Use extreme caution when handling liquid nitrogen.
All centrifugation steps must be carried out at room temperature.

1. Transfer up to 100 mg frozen ground fungal tissue to a 1.5 mL or 2 mL microcentrifuge tube (not provided).

   **Note:** We recommend starting with 50 mg of tissue at first. If results obtained are satisfactory then increase the amount of starting material.

2. Immediately add 600 µL NTL Lysis Buffer. Samples should not be allowed to thaw before NTL Lysis Buffer is added.

   **Note:** NTL Lysis Buffer must be mixed with 2-mercaptoethanol before use. Please see Page 5 for instructions.

3. Vortex vigorously to make sure that all of the clumps have dispersed. RNA cannot be effectively extracted from a clumped sample.

4. Add 140 µL SP Buffer. Vortex to mix thoroughly.

5. Centrifuge at 10,000*g* for 10 minutes at room temperature.

6. Carefully transfer the supernatant of the filtrate to a new 1.5 mL or 2 mL microcentrifuge tube. Do not disturb the pellet or transfer any debris.

   **Tip:** In most cases 600 µL supernatant can easily be removed. This will require 600 µL isopropanol in the next step. Depending on the sample, the volume of supernatant may vary. After transferring to a new tube, measure the volume and add the correct amount of isopropanol in the next step.

7. Add 1 volume isopropanol. Vortex to mix thoroughly.

8. Immediately centrifuge at 10,000*g* for 2 minutes at room temperature. A longer centrifugation does not improve yields.

9. Carefully aspirate and discard the supernatant. Do not disturb the RNA pellet.

10. Invert the microcentrifuge tube on a paper towel for 1 minute to allow residual liquid to drain. Drying the pellet is not necessary.

11. Add 100 µL RB Buffer or Nuclease-free Water heated to 65°C. Vortex at maximum speed to resuspend the pellet. A brief incubation at 65°C may be necessary.

    **Note:** RB Buffer must be mixed with 2-mercaptoethanol before use. Please see Page 5 for instructions.

    **Important:** RB Buffer is recommended to dissolve the RNA pellet at this step, particularly if degradation has been found after elution. RB Buffer contains a strong RNase inhibitor. If it is difficult to dissolve the RNA pellet with RB Buffer, use Nuclease-free Water instead.

12. This step varies depending on the buffer used to resuspend the RNA pellet in Step 11.

    A. For RB Buffer:
       1. Add 250 µL RB Buffer.
       2. Add 350 µL 70% ethanol.
       3. Vortex to mix thoroughly.
       4. Proceed to Step 13.

    B. For Nuclease-free Water:
       1. Add 350 µL RB Buffer.
       2. Add 250 µL 100% ethanol.
       3. Vortex to mix thoroughly.
       4. Proceed to Step 13.

    **Note:** RB Buffer must be mixed with 2-mercaptoethanol before use. Please see Page 5 for instructions.

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

14. Transfer the entire sample from Step 12, including any precipitates that may have formed, to the HiBind® RNA Mini column.

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

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 DNA removal step is required, please continue to the DNase I Digestion Protocol found on Page 15. (See DNase I Digestion Set, (E1091) for more information). If DNase I digestion is not required, proceed to Step 17.

17. Add 500 µL RNA Wash Buffer I.

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

19. Discard the filtrate and the collection tube.

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

21. Add 500 µL RNA Wash Buffer II.

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

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

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 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.

26. Transfer the HiBind® RNA Mini Column to a clean 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. Centrifuge at top speed for 1 minute and store eluted RNA at -70°C.

    **Note:** A second elution into the same tube may be necessary if the expected yield of RNA is >50 µg. Alternatively, RNA may be eluted with a greater volume of Nuclease-free Water. While an additional elution step will increase total RNA yield, the concentration will be lowered since more than 80% of RNA is recovered with the first elution.

## 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 1-12 of the Standard Protocol (Pages 7-8) or Steps 1-16 of the Difficult Sample Types Protocol (Pages 11-13), proceed with the following protocol.

**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*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 for 15 minutes at room temperature.

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

9. Let sit for 2 minutes at room temperature.

10. Centrifuge at 10,000*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 5 for instructions.

13. Centrifuge at 10,000*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 or 2 mL microcentrifuge tube (not supplied).

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 for 1 minute at room temperature.

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

    **Note:** A second elution into the same tube may be necessary if the expected yield of RNA is >50 µg. Alternatively, RNA may be eluted with a greater volume of Nuclease-free Water. While an additional elution step will increase total RNA yield, the concentration will be lowered since more than 80% of RNA is recovered with 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 **1-800-832-8896**.

| Problem | Cause | Solution |
|---|---|---|
| Little or no RNA eluted | RNA remains on the column | • Repeat elution.<br>• Heat Nuclease-free Water to 65°C prior to elution.<br>• Incubate column for 10 minutes prior to centrifugation. |
| | Column is overloaded | Reduce the amount of starting material. |
| **Problem** | **Cause** | **Solution** |
| Clogged column | Incomplete disruption or lysis of fungal tissue | • Completely grind sample in liquid nitrogen.<br>• Increase centrifugation time.<br>• Reduce amount of starting material. |
| Precipitated RNA will not dissolve | High nucleic acid and polysaccharide content | • Reduce amount of starting material. Start with 50-100 mg.<br>• To avoid RNA degradation, use RB Buffer to dissolve the RNA pellet. |
| **Problem** | **Cause** | **Solution** |
| Degraded RNA | Source | • Freeze starting material quickly in liquid nitrogen and store at -70°C without thawing.<br>• Follow protocol closely and work quickly.<br>• Make sure that 2-mercaptoethanol is added to RB and/or NTL Lysis Buffer. |
| | RNase contamination | • Ensure not to introduce RNases during the procedure.<br>• Check buffers for RNase contamination. |
| **Problem** | **Cause** | **Solution** |
| Problem in downstream applications | Salt carryover during elution | • Ensure RNA Wash Buffer II has been diluted with 100% ethanol.<br>• Diluted RNA Wash Buffer II must be stored at room temperature.<br>• Repeat wash step with RNA Wash Buffer II. |
| DNA contamination | Co-purification of DNA | Follow the DNase I Digestion Protocol on Page 15. |
| Low Abs ratios | RNA diluted in acidic buffer or water | Nuclease-free Water is slightly acidic and can lower $A_{260}/A_{280}$ ratios. Use TE buffer to dilute RNA prior to spectrophotometric analysis. |

**Notes:**

**Notes:**

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

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