omega BIO-TEK

innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Plant RNA Kit

R6827-00	5 preps
R6827-01	50 preps
R6827-02	200 preps

September 2014

For Research Use Only

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E.Z.N.A.® Plant RNA Kit

Table of Contents

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Introduction

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 (a silica-based material) in combination with the speed of mini column spin technology. Single or multiple samples can be processed quickly. There is no need for phenol/chloroform extractions and time-consuming steps, such as CsCl gradient ultra-centrifugation 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, poly A+ RNA (mRNA) purification, nuclease protection, and *in vitro* translation.

The E.Z.N.A.® Plant RNA Kit can purify up to 100 µg plant RNA that is >200 nt. Normally, 10-100 mg plant tissue can be processed in a single experiment.

Lysis of cells or tissue occurs under denaturing conditions that inactivate RNases. After the homogenization process, samples are transferred to the HiBind® RNA Mini Column to bind RNA. Cellular debris and other contaminants are removed after three quick wash steps. High-quality RNA is eluted in sterile DEPC Water.

New In this Edition

- The latest edition has been redesigned to enhance readability and protocol quality.
- RWC Wash Buffer is renamed RWF Wash Buffer.

Binding Capacity

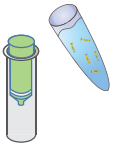
- Each HiBind® RNA Mini Column can bind approximately 100 µg RNA. Using greater than 100 mg plant tissue is not recommended.

Illustrated Protocol

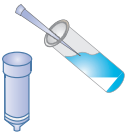
Grind with liquid nitrogen

Add RB Buffer/ RCL Buffer

Lyse



Transfer sample to a Homogenizer Column



Add ethanol /RCB Buffer and transfer sample to HiBind® RNA Mini Column

Wash 3X

Dry

Elute

Kit Contents

Plant RNA Mini Kit	R6827-00	R6827-01	R6827-02
Preparations	5	50	200
HiBind® RNA Mini Columns	5	50	200
gDNA Filter Column	5	50	200
2 mL Collection Tubes	15	150	600
RCL Buffer	5 mL	30 mL	110 mL
RCB Buffer	5 mL	30 mL	110 mL
RB Buffer	5 mL	30 mL	110 mL
RWF Wash Buffer	5 mL	50 mL	2 x 90 mL
RNA Wash Buffer II	2 mL	12 mL	50 mL
DEPC Water	1 mL	10 mL	40 mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® Plant RNA Kit components are guaranteed for at least 24 months from the date of purchase when stored at room temperature. During shipment or storage in cool ambient conditions, precipitates may form in RB Buffer. 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.

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

Preparing Reagents

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

Kit	100% Ethanol to be Added
R6827-00	8 mL
R6827-01	48 mL
R6827-02	200 mL

- Add 20 µL 2-mercaptoethanol per 1 mL RB Buffer. This mixture can be stored for one month at room temperature.

- For Difficult Sample Types protocol (Page 12), add 20 µL 2-mercaptoethanol per 1 mL RCL Buffer. This mixture can be stored for one month 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. However, the E.Z.N.A.® Plant RNA Kit eliminates the use of phenol and avoids this problem. 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.

Expected Yields

Sample yields from 100 mg starting tissue.

Yields obtained with the E.Z.N.A.® Plant RNA Kit	
Arabidopsis	30 µg
Maize Leaves	65 µg
Mustard Leaves	34 µg
Tobacco Leaves	28 µg

E.Z.N.A.® Plant RNA Kit - Standard Protocol

This protocol is suitable for most fresh or frozen tissue samples, thereby allowing efficient recovery of RNA. However, due to the tremendous variation in water and polysaccharide content in plants, sample size should be limited to ≤100 mg. Best results are obtained with young leaves or needles. The method outlined in this protocol will isolate a sufficient amount of RNA for tracks on a standard Northern assay.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000 x *g*
- RNase-free pipet tips and 1.5 mL microcentrifuge tubes
- 100% ethanol
- 2-mercaptoethanol
- Liquid nitrogen
- Optional: Water bath, incubator, or heat block capable of 65°C

Before Starting:

- Prepare RNA Wash Buffer II and RB Buffer according to "Preparing Reagents" section on Page 6

1. Collect tissue in a 1.5 mL microcentrifuge tube (not provided) and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable pestles.

   Note: Disposable Kontes pestles work well and are available for purchase (product no. SSI-1014-39 & SSI-1015-39). One can allow liquid nitrogen to evaporate and store the samples at -70°C for later use. Do not allow samples to thaw. Use disposable pestle only once. Alternatively, a small clean mortar and pestle can be used. The above methods for disrupting plant tissue cannot be replaced with mechanical homogenizers.

2. Transfer up to 100 mg frozen ground plant tissue to a new 1.5 mL microcentrifuge tube.

   Note: We recommend starting with 50 mg of tissue at first. If results obtained are satisfactory, you may start increasing the amount of starting material. **Samples should not be allowed to thaw before the addition of RB Buffer in Step 3.**

3. Immediately add 500 µL RB Buffer. Vortex at maximum speed to mix thoroughly.

   Note: RB Buffer must be mixed with 2-mercaptoethanol before use. Please see Page 6 for instructions. Ensure that all the samples are completely suspended and that there are no clumps in the solution. Clumps will result in low yields.

4. Insert a gDNA Filter Column into a 2 mL Collection Tube.

5. Transfer the lysate to a gDNA Filter Column.

6. Centrifuge at 14,000 x *g* for 5 minutes at room temperature.

7. Transfer cleared lysate to a new 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the insoluble pellet. Measure the volume of the lysate.

8. Add 0.5 volume absolute ethanol. Vortex at maximum speed for 20 seconds. A precipitate may form at this point; it will not interfere with DNA isolation. Passing the mixture through a needle using a syringe or by pipetting up and down 10-15 times may break up the precipitates.

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

10. Transfer 700 µL sample, including any precipitates that may have formed, to the HiBind® RNA Mini Column.

11. Centrifuge at 12,000 x *g* for 1 minute at room temperature.

12. Discard 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 16. (See DNase I Digestion Set, Cat # E1091 for more information). If DNase I digestion is not required, proceed to Step 14.

14. Add 400 µL RWF Wash Buffer.

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

16. Discard the filtrate and the collection tube.

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

18. Add 500 µL RNA Wash Buffer II.

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

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

20. Discard the filtrate and reuse collection tube.

21. Add 500 µL RNA Wash Buffer II.

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

23. Discard the filtrate and reuse collection tube.

24. Centrifuge the empty HiBind® RNA 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.

25. Transfer the HiBind® RNA Mini Column to a clean 1.5 mL microcentrifuge tube.

26. Add 50-100 µL DEPC Water.

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

27. 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 DEPC Water to 65°C before adding to the column.
    - Let sit at room temperature for 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh DEPC 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® Plant RNA Kit - Difficult Sample Protocol

In certain plant samples, RNA isolation can be difficult due to their large amount of polysaccharides and phenolic compounds. This protocol involves a simple and rapid precipitation that will remove much of these compounds. **Use this protocol when the standard protocol (Page 8) results in low RNA yields.**

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 14,000 x *g*
- RNase-free pipet tips and 1.5 mL microcentrifuge tubes
- Water bath, incubator, or heat block capable of 55°C
- 100% ethanol
- 2-mercaptoethanol
- Liquid nitrogen

Before Starting:

- Prepare RNA Wash Buffer II, RCL Buffer according to "Preparing Reagents" section on Page 6
- Set water bath, incubator, or heat block to 55°C

1. Collect tissue in a 1.5 mL microcentrifuge tube (not provided) and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable pestles.

   Note: Disposable Kontes pestles work well and are available for purchase (product no. SSI-1014-39 & SSI-1015-39). One can allow liquid nitrogen to evaporate and store the samples at -70°C for later use. Do not allow samples to thaw. Use disposable pestle only once. Alternatively, a small clean mortar and pestle can be used. The above methods for disrupting plant tissue cannot be replaced with mechanical homogenizers.

2. Transfer up to 100 mg frozen ground plant tissue to a new 1.5 mL microcentrifuge tube.

   Note: We recommend starting with 50 mg of tissue at first. If results obtained are satisfactory, you may start increasing the amount of starting material. **Samples should not be allowed to thaw before the addition of RCL Buffer in Step 3.**

3. Immediately add 500 µL RCL Buffer. Vortex at maximum speed to mix thoroughly.

   Note: RCL Buffer must be mixed with 2-mercaptoethanol before use. Please see Page 6 for instructions. Ensure that all the samples are completely suspended and that there are no clumps in the solution. Clumps will result in low yields.

4. Incubate at 55°C for 1-3 minutes.

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

6. Transfer cleared lysate directly into a gDNA Filter Column in 2 ml collection tube and centrifuge at 14,000 x g for 2 minutes at room temperature.

7. Add 1 volume RCB Buffer to the flow-through . Vortex at maximum speed for 20 seconds.

   Note: In most cases 450 µl cleared lysate can easily be removed. Add 450 µL RCB Buffer. Depending on the sample, the volume of the cleared lysate will vary. After transferring to a fresh tube, measure the volume and add the correct amount of Buffer RCB.

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

9. Transfer 700 µL sample, including any precipitates that may have formed, to the HiBind® RNA Mini Column.

10. Centrifuge at 12,000 x *g* for 1 minute at room temperature.

11. Discard the filtrate and reuse the Collection Tube.

12. Repeat Steps 9-11 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 16. (See DNase I Digestion Set, Cat # E1091 for more information). If DNase I digestion is not required, proceed to Step 13.

13. Add 400 µL RWF Wash Buffer.

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

15. Discard the filtrate and the collection tube.

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

17. Add 500 µL RNA Wash Buffer II.

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

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

19. Discard the filtrate and reuse collection tube.

20. Add 500 µL RNA Wash Buffer II.

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

22. Discard the filtrate and reuse collection tube.

23. Centrifuge at maximum speed for 1 minute 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.

25. Add 50-100 µL DEPC Water.

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

26. 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 DEPC Water to 65°C before adding to the column.
    - Let sit at room temperature for 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh DEPC 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. (See DNase I Digestion Set, Cat# E1091 for further information).

After completing Steps 1-13 of the Standard Protocol (Pages 8-9) or Steps 1-12 of the Difficult Samples Protocol, 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 RWF Wash Buffer 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 RWF Wash Buffer 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 ethanol before use. Please see Page 6 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 50-100 µL DEPC Water.

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

19. 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 DEPC Water to 65°C before adding to the column.
    - Let sit at room temperature for 5 minutes.
    - Increase the elution volume.
    - Repeat the elution step with fresh DEPC 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 toubleshoot any problems that may arise. For further assistance, please contact the technical support staff , toll free, **at 1-800-832-8896.**

Possible Problems and Suggestions

Problem	Cause	Solution
Little or no RNA eluted	RNA remains on the column	Repeat elution step.
		Heat DEPC Water to 65°C prior to elution.
	Column is overloaded	Reduce quantity of starting material.
Problem	Cause	Solution
Clogged column	Incomplete homogenization	Completely homogenize sample.
		Increase centrifugation time.
		Reduce amount of starting material
Problem	Cause	Solution
Degraded RNA	Starting sample problems	Freeze starting material quickly in liquid nitrogen.
		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 carryover during elution	Ensure RNA Wash Buffer II has been diluted with 4 volumes 100% ethanol as indicated on bottle.
		RNA Wash Buffer II must be stored and used at room temperature.
		Repeat wash with RNA Wash Buffer II.
Problem	Cause	Solution
DNA contamination	DNA contamination	Perform the optional DNase I Digestion Protocol on Page 16.
Problem	Cause	Solution
Low Abs ratios	RNA diluted in acidic buffer or water	DEPC Water is acidic and can dramatically lower Abs_{260} values. Use TE buffer to dilute RNA prior to spectrophotometric analysis.

Ordering Information

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

Product	Part Number
RB Buffer (100 mL)	PR026
RWF Wash Buffer (100 mL)	PR104
RNA Wash Buffer II (25 mL)	PR031
DEPC Water (100 mL)	PR032
2 mL Collection Tubes	SS1-1370-00
RNase-free DNase Set (50 preps)	E1091
Proteinase K Solution	AC115-AC116

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

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innovations in nucleic acid isolation

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