OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

E.Z.N.A.® Soil RNA Midi Kit

R6826-00	2 preps
R6826-01	10 preps
R6826-01	25 preps

April 2015

For research use only.Not intended for diagnostic testing.

E.Z.N.A.® Soil RNA Midi Kit

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Introduction

The E.Z.N.A.® Soil RNA Midi Kit is designed to isolate high-quality total RNA from soil samples typically containing humic acid and inhibitors of RT-PCR. This kit uses a novel and proprietary method to isolate total RNA from a variety of environmental samples. Soil samples are homogenized and extracted with phenol/chloroform. A specially formulated suspension buffer is added to remove coloration and inhibitors. By using an innovative DNA Clearance Maxi Column, DNA is effectively removed without the need for DNase digestion. The filtrate containing the RNA is adjusted with ethanol and transferred to the HiBind® RNA Midi Column which binds total RNA while cellular debris and other contaminants are effectively washed away. High-quality RNA is eluted in RNA Elution Buffer. The entire procedure can be completed in under one hour.

This kit has been successfully used to isolated RNA from soil samples collected from forest, riverbed, grassland, garden and cultivated fields. Isolated RNA can be used for most downstream applications including RT-PCR, Northern blot, and more.

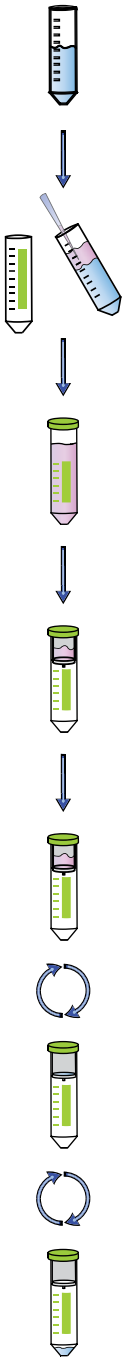
Binding Capacity

Each HiBind® RNA Midi Column can bind approximately 1 mg RNA.

New in this Edition:

- RNA Binding Buffer has been replaced with RBB Buffer.
- A new buffer system has been developed to enhance color removal due to humic acids and reduce inhibitors thereby increasing RNA quality.

Illustrated Protocol



Homogenize soil sample with lysis buffer, glass beads, HTR2 Reagent, and phenol/chloroform

Transfer Aqueous Phase

Add Binding Buffer

Pass through DNA Clearance Maxi Column

Add Binding Buffer
Bind to HiBind® RNA Midi Column

Wash 3X

Dry

Elute

Kit Contents

Product	R6826-00	R6826-01	R6826-02
Purifications	2	10	25
DNA Clearance Maxi Column	2	10	25
50 mL Collection Tubes	2	10	25
HiBind® RNA Midi Column	2	10	25
15 mL Collection Tubes	2	10	25
Glass Beads 1	2.5 g	12 g	30 g
Glass Beads 2	2.5 g	12 g	30 g
SLX Buffer	10 mL	50 mL	120 mL
HTR2 Reagent	2 mL	5 mL	12 mL
SP2 Buffer	2 mL	5 mL	12 mL
XP2 Buffer	10 mL	50 mL	120 mL
RBB Buffer	20 mL	100 mL	250 mL
RWF Wash Buffer	7 mL	35 mL	80 mL
RNA Wash Buffer II	3 mL	15 mL	40 mL
RNA Elution Buffer	2 mL	10 mL	25 mL
User Manual	✓	✓	✓

Storage and Stability

All of the E.Z.N.A.® Soil RNA Midi Kit components are guaranteed for at least 24 months from the date of purchase when stored at room temperature.

Preparing Reagents

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

Kit	100% Ethanol to be Added
R6826-00	12 mL
R6826-01	60 mL
R6826-02	160 mL

2. Prepare water-saturated phenol as follows:

   Place the solid phenol in a water bath preset at 75°C until phenol is completely dissolved. Add an equal volume of molecular biology grade water and mix thoroughly by shaking. Store the solution at room temperature for 4 hours to overnight until the water phase (upper phase) and phenol phase (lower phase) are clearly separated. Remove the water phase with a transfer pipette.

Storage and Quantification of RNA

Storage of RNA

Purified RNA is stable for more than a year when stored at -70°C.

Quantification 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. If it is necessary to dilute RNA sample, use a buffer with neutral pH. 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 absorbance readings of DNA or RNA.

RNA Quality

It is highly recommended that RNA quality be determined prior to all analysis. The quality of RNA can be best assessed by denaturing agarose gel electrophoresis and ethidium bromide staining. Two sharp bands, corresponding to the 28S and 18S (23S and 16S for bacteria) ribosomal RNA, should be visible on the gel. If these bands appear as smear towards smaller size, it is likely that RNA has undergone degradation during preparation, handling, or storage.

E.Z.N.A.® Soil RNA Midi Kit Protocol

Materials and Equipment to be Supplied by User:

- 100% ethanol
- Water-saturated phenol (use molecular biology grade water)
- Chloroform
- Refrigerated centrifuge with 15 mL and 50 mL centrifuge tube adaptors capable of at least 4,000 x *g*
- Vortexer
- Nuclease-free pipette tips
- Nuclease-free 15 mL and 50 mL centrifuge tubes
- Optional: RNase-free DNase I Digestion Kit (Cat# E1091)

Before Starting:

- Prepare RNA Wash Buffer II and water-saturated phenol according to the "Preparing Reagents" section on Page 5

1. Add 2 g soil sample, 1 g Glass Beads 1, and 1 g Glass Beads 2 to a 50 mL centrifuge tube (not supplied).

2. Add 4 mL SLX Buffer and 400 µL HTR2 Reagent. Vortex for 1 minute to mix thoroughly.

   Note: Shake the HTR2 Reagent to resuspend before use.

3. Add 4 mL water-saturated phenol. Vortex at maximum speed for 10 minutes.

   Note: For optimal results, use a bead mixer such as GenoGrinder.

4. Add 4 mL chloroform. Vortex at maximum speed for 1 minute.

5. Centrifuge at 4,000 x *g* for 10 minutes at 4°C to separate the aqueous and organic phases.

   Note: The sample should separate into 3 phases: an upper aqueous phase (often light brown) which contains RNA and DNA; a light-colored organic interphase consists of phenol/chloroform; and a lower insoluble phase of soil and debris.

6. Immediately transfer the upper aqueous phase (~4 mL) to a new 50 mL centrifuge tube (not supplied). Take care not to disturb the organic layer.

7. Add 0.1 volume SP2 Buffer (~400 µL) and 1 volume XP2 Buffer (~4 mL). Vortex to mix thoroughly.

8. Transfer the sample to a DNA Clearance Maxi Column inserted in a 50 mL Collection Tube (provided).

9. Centrifuge at 4,000 x *g* for 5 minutes at 4°C.

10. Discard the DNA Clearance Maxi Column only. ***Save the filtrate***.

11. Add 1 volume RBB Buffer to the filtrate. Invert 10-30 times to mix thoroughly.

12. Transfer 4 mL sample to the HiBind® RNA Midi Column inserted in a 15 mL Collection Tube (provided).

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

14. Discard the filtrate and reuse the Collection Tube.

15. Repeat Steps 12-14 until all 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 HiBind® 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 11. (See DNase I Digestion Set, (E1091) for more information). If DNase I digestion is not required, proceed to Step 16.

16. Add 3 mL RWF Wash Buffer.

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

18. Discard the filtrate and reuse the Collection Tube.

19. Add 3 mL RNA Wash Buffer II.

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

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

21. Discard the filtrate and reuse the Collection Tube.

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

23. Centrifuge at maximum speed for 20 minutes to completely dry the HiBind® RNA Midi Column.

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

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

25. Add 400-500 µL RNA Elution Buffer.

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

26. Let sit at room temperature for 3 minutes.

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

28. Store eluted RNA at -70°C.

E.Z.N.A.® Soil RNA Mini Kit - DNase I Digestion Protocol

Since the HiBind® matrix of the HiBind® 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# E1091-02 for further information).

After completing Steps 1-15 of the E.Z.N.A.® Soil RNA Midi Kit Protocol, proceed with the following protocol.

User Supplied Material:

- DNase I Digestion Set (E1091-02)

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	294 µL
RNase-free DNase I (20 Kunitz/µL)	6 µL
Total Volume	300 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 RWF Wash Buffer.

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

4. Discard the filtrate and reuse the Collection Tube.

5. Add 300 µL DNase I digestion mixture directly onto the surface of the membrane of the HiBind® RNA Midi 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 Midi Column.

6. Let sit at room temperature for 15 minutes.

7. Add 1.5 mL RWF Wash Buffer.

8. Let sit at room temperature for 2 minutes.

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

10. 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 ethanol before use. Please see Page 5 for instructions.

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

13. Discard the filtrate and reuse the Collection Tube.

14. Repeat Steps 11-13 for a second RNA Wash Buffer II wash step.

15. Centrifuge at maximum speed for 20 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.

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

17. Add 400-500 µL RNA Elution Buffer.

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

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

19. Store eluted RNA at -70°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **(800-832-8896).**

Possible Problems and Suggestions

Problem	Cause	Solution
Low RNA yield	Incompletely sample lysis	Increase the vortex speed and duration at Steps 1 and 3. Use a bead beater if available.
	Column is not dried completely before elution	Make sure the empty column is centrifuged for at least 20 minutes before elution.
	RNA Wash Buffer II is not prepared correctly with ethanol	Ensure RNA Wash Buffer II is diluted with 4 volumes of 100% ethanol as indicated on the bottle.
Degraded RNA	Starting sample Problems	Always use fresh soil sample when possible.
	RNase contamination	Ensure not to introduce RNase during the procedure.
		Check buffers for RNase contamination.
Problem with downstream applications	Salt carryover during elution	Ensure RNA Wash Buffer II is diluted with 4 volumes of 100% ethanol as indicated on the bottle.
		RNA Wash Buffer II must be stored and used at room temperature.
DNA contamination	Sample has very high DNA content	Digest with RNase-free DNase and inactivate DNase by incubating at 65°C for 5 minutes in the presence of EDTA.
Problem	Cause	Solution
Low Abs ratios	RNA contains humic acid	Increase HTR2 Reagent volume in Step 2.

Ordering Information

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

Buffer (Size)	Part Number
RNA Wash Buffer II (25 mL)	PDR046

Notes: