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Introduction

E.Z.N.A.™ Fungal RNA Kit provides a rapid and reliable method for isolation of total RNA from a wide variety of fungal samples.The kit does not require the use of cumbersome or expensive shredding/homogenizing accessories as an attempt to shear viscous fungal Lysates. Rather, the 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 spin columns, this 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 of tissue or 100 cells.Typical yields are shown in Table 1. E.Z.N.A.™ Fungal RNA Kits are ideal for processing multiple fungal samples parallel in 1 hour. 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 A260/A280 ratios of 1.8-20 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 Kits	
Acremonium crysogenum	50 mg
Fusarium avenaceu	37 mg
Mushrooms	43 mg

New in this edition:

The new introduced capped V-Spin column (R6840) virtually assures the elimination of potential contamination during the ecperiment.

Storage and Stability

All components of the E.Z.N.A.™ Fungal RNA Kit are stable for at least 24 months from date of purchase when stored at 22°C-25°C. During shipment, or storage in cool ambient conditions, precipitates may form in RB. It is possible to dissolve such deposits by warming the solution at 37°C, though we have found that they do not interfere with overall performance.

Binding Capacity

Each HiBind RNA column can bind approximately 100 mg RNA. Using greater than 250 mg fungal tissue in many cases will not drama tical improve yields and sometimes has adverse affects.

Kit Contents

Product Number	R6640-00 R6840-00	R6640-01 R6840-01	R6640-02 R6840-02
HiBind™ RNA Columns	5	50	200
2 ml Collection Tubes	10	100	400
RFL	5 ml	60 ml	2 x 125 ml
Buffer SP	2 ml	10 ml	40 ml
Buffer RB	5 ml	30 ml	110 ml
RNA Wash Buffer II	5 ml	12 ml	2 x 25 ml
RNA wash Buffer I	5 ml	45 ml	2 x 80 ml
DEPC Water	1 ml	5ml	20 ml
Instruction Booklet	1	1	1

Buffer RB contains a chaotropic salt. Use gloves and protective eyeware when handling this solution.

Before Starting

- Please read the entire booklet to become familiar with the E.Z.N.A.™ Fungal RNA procedure.
- Dilute Wash Buffer Concentrate with ethanol as follows and **store at room temperature**.

R6640-00, R6840-00 Add 20 ml absolute (96%-100%) ethanol.

R6640-01, R6840-01 Add 48 ml absolute (96%-100%) ethanol to each bottle.

R6640-02, R6840-02 Add 100 ml absolute (96%-100%) ethanol to each bottle.

Working with RNA

Please take a few minutes to read this booklet thoroughly to become familiar with the protocol. Prepare all materials required before starting to minimize RNA degradation.

- Whenever working with RNA, always wear latex gloves to minimize RNase contamination. Change gloves frequently. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.
- During the procedure work carefully but quickly.
- Under cool ambient conditions, crystals may form in Buffer RB.This is normal and the bottle may be warmed to redissolve the salt.
- 2-mercaptoethanol (ß-mercaptoethanol) is key in denaturing endogenous RNases and must be added to an aliquot of Buffer RB and Buffer RPL before use. Add 20 µl of 2-mercaptoethanol per 1 ml of Buffer RB or RFL. This mixture can be stored for 1 week at room temperature.

E.Z.N.A.™ Fungal RNA Protocol

Materials to be provided by user:

- Microcentrifuge capable of 10,000 x g
- Nuclease-free microfuge tubes
- 2-mercaptoethanol
- Absolute (96%-100%) ethanol
- Isopropyl alcohol (isopropanol)
- Liquid nitrogen for freezing/disrupting samples
- Preheat an aliquot (100 µl per sample) of DEPC-treated water at 65°C.

NOTE: Use extreme caution when handling liquid nitrogen.

This protocol is suitable for most fresh or frozen tissue samples allowing efficient recovery of RNA. However, due to the tremendous variation in water and polysaccharide content of plants, sample size should be limited to ≤200 mg. Best results are obtained with young leaves or needles.The method isolates sufficient RNA for a few tracks on a standard Northern assay.
Wearing latex disposable gloves collect tissue in a1.5-ml or 2-ml microfuge tube and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable homogenization pestles (available from Omega Bio-tek, Cat# SSI-1015-39) or equivalent. Alternatively, one can allow liquid nitrogen to evaporate and then store samples at -70°C for later use. Do not allow samples to thaw. Use disposable pestles 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.
Note that all centrifugation steps must be carried out at room temperature.

1. **Collect frozen ground Fungal tissue (up to 200 mg) in a microfuge tube and immediately add 600 µl Buffer RFL/2-mercaptoethanol**. We recommend starting with 50 mg tissue at first. If results obtained are satisfactory increase amount of starting material. Add 20 µl 2-mercaptoethanol per 1ml of Buffer RFL and then add 600 µl to the sample. **Samples should not be allowed to thaw before Buffer RPL/2-mercaptoethanol is added.** Vortex vigorously to make sure that all clumps are dispersed. RNA cannot be effectively extracted from clumped tissue.

Note: **Add 20 µl 2-mercaptoethanol per 1 ml of Buffer RPL before use**. This mixture can be made and stored at room temperature for 1 week.

TIP: As a guide, a 2-cm diameter leaf square weighs approximately 100 mg. Process in sets of four to six tubes: fill all tubes with liquid nitrogen, grind, add Buffer RPL/2-mercaptoethanol, and continue to step 2 before starting another set. Centrifuge all tubes simultaneously (step 2 below).We recommend starting with 50-100 mg tissue at first to ensure optimal results. Adjust amount of starting material according to results obtained.

2. **Add 140 µl Buffer SP and vortex thoroughly to mix. Centrifuge at 10,000 x g for 10 min at room temperature**.

3. **Carefully aspirate cleared lysate to a new microfuge tube making sure not to disturb the pellet or transfer any debris. Add one volume isopropanol and vortex to precipitate RNA**. This step removes much of the polysaccharide content and improves spin-column performance by increasing RNA binding capacity (and therefore yield) in the steps that follow. No incubation is required after addition of isopropanol.

   TIP: In most cases 600 µl supernatant can easily be removed. This will require 600 µl isopropanol. Note that depending on the sample, the volume of supernatant may vary. After transferring to a fresh tube, measure the volume and add the correct amount of isopropanol.

4. **Immediately centrifuge at 10,000 x g for 2 min at room temperature to pellet RNA**. A longer centrifugation does not improve yields.

5. **Carefully aspirate or decant the supernatant and discard making sure not to dislodge the RNA pellet**. Invert the microfuge tube on a paper towel for 1 min to allow residual liquid to drain. Drying the pellet is not necessary.

6. **Add 100 µl of sterile DEPC-treated water (supplied) pre-heated to 65°C and vortex to resuspend the pellet**. A brief incubation at 65°C may be necessary to effectively dissolve the RNA.

   Important: RB buffer also can be used to dissolve the RNA pellet at this step, especially when the degradation was found after the elution. RB buffer contains strong RNase inhibitor.

7. **If water is used in step 6, add 350 µl Buffer RB/2-mercaptoethanol followed by 250 µl absolute ethanol. If RB Buffer is used in step 6, add 250 µl RB and followed by 350 µl 70% ethanol**. Vortex thoroughly to mix. This will adjust binding conditions prior to loading the HiBind® RNA column.

   Note: Add 20 µl 2-mercaptoethanol per 1 ml of Buffer RB before use. This mixture can be made and stored at room temperature for 1 week.

8. **Apply the entire sample, including any precipitates that may form to an HiBind® RNA spin column** assembled in a clean 2 ml collecting tube (supplied). Centrifuge at 10,000 x g for 30 sec at room temperature. Discard the flow-through liquid and place the column back into the collecting tube.

9. **Add 750 µl RNA Wash Buffer I and centrifuge at 10,000 x g for 30 sec**. Discard both flow-through liquid and collecting tube.

10. **Place column in a clean 2ml collection tube (supplied), and add 500 µl Wash Buffer II diluted with ethanol**. Centrifuge at 10,000 x g for 30 sec at room temperature and discard flow-through. Reuse the collection tube in step 10.

    Note: Wash Buffer II Concentrate must be diluted with absolute ethanol before use. Refer to label on bottle for directions.

11. **Wash column with a second 500 µl of Wash Buffer II as in step 9**. Centrifuge and discard flow-through. Then with the collection tube empty, centrifuge the spin cartridge for 1 min at full speed to completely dry the HiBind™ matrix.

12. **Elution of RNA. Transfer the column to a clean 1.5 ml microfuge tube (not supplied with kit) and elute the RNA with 50-100 µl of DEPC-treated water (supplied with kit)**. Make sure to add water directly onto column matrix. Centrifuge 1 min at maximum speed. A second elution into the same tube may be necessary if the expected yield of RNA >50 µg.

    Note: RNA may be eluted with a greater volume of water. While additional elation increase total RNA yield, the concentration will be lowered since more than 80% of RNA is recovered with the first elution.

RNA Quality

It is highly recommended that RNA quality be determined prior to all analyses. The quality of RNA can be assessed by denaturing agarose gel electrophoresis and ethidium bromide staining. Several sharp bands should appear on the gel. These are the 28S and 18S ribosomal RNA bands as well as certain populations of mRNA and possibly viral RNA bands. If these bands smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling, or storage. RNA molecules less than 200 bases in length do not efficiently bind the HiBind matrix, thus the method enriches high quality RNA. Since no RNA extraction procedure can completely remove genomic DNA. For sensitive work (such as RT-PCR or differential display) either on-membrane DNase I digestion treatment or after elution DNase I digestion will be needed. For modified protocols for DNase I digestion, call our technical staff at 800.832.8896 for assistance

Trouble Shooting Guide

Problem	Cause	Suggestion
Little or no RNA eluted	RNA remains on the column	! Repeat elution. ! Pre-heat DEPC-water to 70° C prior to elution. ! Incubate column for 10 min with water prior to centrifugation.
	Column is overloaded	! Reduce quantity of starting material.
Clogged column	Incomplete disruption or lysis of plant tissue.	! Completely disrupt sample in liquid nitrogen. ! Increase centrifugation time. ! Reduce amount of starting material
Precipitated RNA will not dissolve.	High nucleic acid and polysaccharide content.	! Reduce amount of starting material. Generally it is best to start with 50-100 mg at first. ! To avoid RNA degradation, do not increase incubation time for resuspension.
Degraded RNA	Source	! Freeze starting material quickly in liquid nitrogen and store at -70°C without thawing. ! Follow protocol closely, and work quickly. ! Make sure that 2-mercaptoethanol is added to Buffer RPL.
	RNase contamination	! Ensure not to introduce RNase during the procedure. ! Check buffers for RNase contamination.
Problem in downstream applications	Salt carry-over during elution	! Ensure Wash Buffer II has been diluted with 100% ethanol as indicated on bottle. ! Diluted Wash Buffer II must be stored at room temperature. ! Repeat wash with Wash Buffer II.
DNA contamination	Co-purification of DNA	! Digest with RNase-free DNase and inactivate at 75°C for 5 min.
Low Abs ratios	RNA diluted in acidic buffer or water	! DEPC-treated water is acidic and can dramatically lower Abs260 values. Use TE buffer (pH 8) to dilute RNA prior to spec analysis.

Ordering Information

Product Number	Product Name	Description
R6634-01/02 R6834-01/02	E.Z.N.A.™ Total RNA Kit	Total RNA isolation from animal cells or tissues.
R6614-01/02 R6814-01/02	E.Z.N.A.™ Blood RNA Kit	Total RNA Isolation from blood samples
R6627-01/02 R6827-01/02	E.Z.N.A.™ Plant RNA Kit	Total RNA Isolation from plant samples
R6640-01/02 R6840-01/02	E.Z.N.A.™ Fungal RNA Kit	Total RNA Isolation from fungal samples
R6670-01/02 R6870-01/02	E.Z.N.A.™ Yeast RNA Kit	Total RNA Isolation from yeast samples
R6850-01/02 R6950-01/02	E.Z.N.A.™ Bacterial RNA Kit	Total RNA Isolation from yeast samples
R6675-01/02 R6875-01/02	E.Z.N.A.™ Mollusc RNA Kit	Total RNA Isolation from mollusc, invertebrates samples.
R6664-01/02	E.Z.N.A.™ Total RNA Midi Kit	Total RNA isolation from animal cells or tissues
R6693-01/02	E.Z.N.A.™ Total RNA Maxi Kit	Total RNA isolation from animal cells or tissues
R6615-01/02	E.Z.N.A.™ Blood RNA Midi Kit	Total RNA isolation from blood samples
R6616-01/02	E.Z.N.A.™ Blood RNA Maxi Kit	Total RNA isolation from blood samples
R6628-01/02	E.Z.N.A.™ Plant RNA Midi Kit	Total RNA isolation from plant samples
R6511-01/02	mRNA Enrichment kit	mRNA isolation
R6830-01/02	RNA-Solv™ reagent	Single reagent for total RNA isolation
R6248-01/02 R6249-01/02	E.Z.N.A.™ RNA Probe purification kit	RNA Probe purification
R6376-01/02	E.Z.N.A.™ Poly-Gel RNA Isolation Kit	Isolate RNA from poly-acrylamide gel
R6500-01/02	E.Z.N.A.™ Oligo (dT) Cellulose	High capacity oligo(dT) cellulose
E1091	RNase-free DNase I set	DNase I set for on-column DNase digestion