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EasyRNA™ Fungal RNA Mini Kit

(Cat# K1419-50, -250; Store at Multiple Temperatures)

I. Introduction:

BioVision's EasyRNA™ Fungal RNA Mini 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, then method involves a simple and rapid precipitation step for removal of much of the polysaccharides and phenolic compounds commonly found in fungal tissues. In combination with RNA spin columns, 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 of tissue or 100 cells. The procedure involves no organic extractions, thus reducing plastic waste and hands-on time. Fungal RNA Kits are ideal for processing multiple fungal samples in parallel in 1 hour. Purified RNA has A_{260}/A_{280} ratios of 1.8-20 and is suitable for RT-PCR, Northern Analysis, Differential display, Poly A+RNA selection.

II. Sample Type:

For fast and efficient isolation of total RNA from a wide variety of fungal samples.

III. Kit Contents:

Components	K1419-50 50 Preparations	K1419-250 250 Preparations	Part Number
Buffer FLY	30 mL	135 mL	K1419-XX-1
Buffer RB	30 mL	135 mL	K1419-XX-2
RNA Wash Buffer*	20 mL	3 x 24 mL	K1419-XX-3
DEPC-Treated ddH_2O	10 mL	30 mL	K1419-XX-4
DNase Stop Buffer**	2.4 mL	12 mL	K1419-XX-5
RNA Columns with Tubes	50	250	K1419-XX-6
Microcentrifuge Tubes	100	500	K1419-XX-7

**Add 80 mL (K1419-50) or 96 mL (K1419-250) 100% ethanol to RNA Wash Buffer before use. **Add 9.6 mL (K1419-50) or 48 mL (K1419-250) 100% ethanol to DNase Stop Buffer before use. DNase I and RNase Inhibitor not supplied.*

IV. User Supplied Reagents and Equipment:

- Tabletop microcentrifuge
- 1.5 mL sterile tubes
- Vacuum manifold if use vacuum protocol
- 100% ethanol
- β-mercaptoethanol
- ***Optional: DNase I, DNase Buffer***

V. Shipment and Storage:

All the reagents are shipped at room temperature. DNase I (optional) should be stored at -20°C. All other components can be stored at room temperature. All kit components are guaranteed for 12 months from the date of purchasing.

VI. Reagent Preparation and Storage Conditions:

Prepare all components and get all necessary materials ready by examining the protocol and become familiar with each step.

- Add 1% volume of β-mercaptoethanol to Buffer LY before use and store at 4°C.
- Add 80 mL (K1419-50) or 96 mL (K1419-250) 100% ethanol to RNA Wash Buffer before use. Add 9.6 mL (K1419-50) or 48 mL (K1419-250) 100% ethanol to DNase Stop Buffer before use. The final ethanol is 80% (v/v).

VII. EasyRNA™ Fungal RNA Protocol:

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 fungal sample, sample size should be limited to #100 mg. The method isolates sufficient RNA for a few tracks on a standard Northern assay. Wearing latex disposable gloves, collect tissue in a 1.5 mL or 2.0 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 or equivalent. Alternatively, one can allow liquid nitrogen to evaporate and then store samples at -70oC 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 fungal tissue cannot be replaced with mechanical homogenizers. *Note: All centrifugation steps must be carried out at room temperature.*

1. Weigh **30-100 mg fugal tissue** in a 2 mL tube. Freeze the plant tissue in liquid nitrogen and grind using a rotor starter.
2. Collect frozen ground fungal sample (up to 100 mg) in a microfuge tube and immediately **add 500 µL Buffer FLY/ß-mercaptoethanol.** We recommend starting with 50 mg tissue at first. If results obtained are satisfactory increase amount of starting material. Vortex vigorously to make sure that all the clumps are dispersed. RNA cannot be effectively extracted from clumped sample. *Note: Add **20 µL ß-mercaptoethanol** per 1 ml of Buffer FLY before use. Samples should not be allowed to thaw before Buffer FLY/ ß- mercaptoethanol is added.*
3. Centrifuge at 13,000g for 5 min at room temperature.
4. Carefully transfer the supernatant of the flow-through fraction to a new 1.5 mL microfuge tube, making sure not to disturb the pellet or transfer any debris. **Add 0.5 volume absolute ethanol** and mix by vortexing.
5. Apply the entire sample, including any precipitates that may form to a ezBind RNA spin column assembled in a clean 2 mL collecting tube (supplied). Close the cap gently. Centrifuge at 10,000 g for 30 s at room temperature. Discard the flow-through liquid and place the column back into the collecting tube.
6. Add **500 µL Buffer RB**, close the tube gently. Centrifuge at 10,000g for 30 s. Discard both flow-through liquid and collecting tube.
7. Place column in a clean 2mL collection tube (supplied), and **add 500 µL RNA Wash Buffer** diluted with ethanol. Close the column gently, Centrifuge at 10,000 g for 30 s at room temperature and discard flow-through. Re-use the collection tube in step 8.
8. Wash column with a second **500 µL of RNA Wash Buffer** by repeating step 7. 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 matrix.

9. Elution of RNA. Transfer the column to a clean 1.5 mL microfuge tube 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 elution's increase total RNA yield, the concentration will be lowered since more than 80% of RNA is recovered with the first elution. No RNA extraction procedure can completely remove genomic DNA. For sensitive work (such as RT-PCR or differential display) we suggest that you treat the eluted RNA with RNase-free DNase. Also for RT-PCR, use intron spanning primers that allow easy identification of DNA-contamination. A control PCR reaction containing the RNA as template will also allow detection of DNA contamination.*

DNase digestion Protocol (Optional, not provided in the kit):

a. For each RNA column, prepare the **DNase I digestion reaction mix** as follows:

DNase I Digestion Buffer	47 µL
RNase-free DNase I (2 U/µL)	3 µL
Total volume	50 µL

Note: DNase I is very sensitive and prone to physical denaturing. So, do not vortex the DNase I mixture. Mix gently by inverting the tube. Prepare the fresh DNase I digestion mixture before RNA isolation. DNase I digestion buffer is supplied with RNase-free DNase I. Standard DNase buffers are not compatible with on-membrane DNase digestion.

b) Pipet 50 µL of the DNase I digestion reaction mix directly onto the surface of the ezBind RNA resin in each column. Make sure to pipet the DNase I digestion mixture directly onto the membrane.

c) Incubate at room temperature(25-30oC) for 15 minutes.

d) Add **200 µL DNase Stop Buffer** onto the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through. Add **300 µL RNA Wash Buffer** to the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through.

VIII. Quantization and Storage of RNA:

To determine the concentration and purity of RNA, measure absorbance at 260 nm and 280 nm in a spectrophotometer. 1 O.D. unit measured at 260 nm corresponds to 40 µg of RNA per mL. The ratio of A_{260}/A_{280} of pure nucleic acids is 2.0, while for pure protein it is approximately 0.6. A ratio of 1.8-2.0 corresponds to 90%-100% pure nucleic acid. (Phenol has an absorbance maximum at 275 nm and can interfere with spectrophotometric analysis of DNA or RNA. However, the Fungal RNA Kit eliminates the use of phenol and avoids this problem. Store RNA samples at -70^{o}C in water. Under such conditions RNA prepared with this system is stable for more than a year.

FOR RESEARCH USE ONLY! Not to be used on humans.