

Fungal RNA Miniprep Kit

(BW-R6618)

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Kit Contents

Catalog#	BW-R6618-00	BW-R6618-01	BW-R6618-02
Preps	4	50	250
RNA Columns	4	50	250
DNA Clearance Columns	4	50	250
2 mL Collection Tubes	8	100	500
1.5 mL RNase-free Microfuge Tubes	4	50	250
Buffer FLY	2 mL	30 mL	135 mL
RNA Wash Buffer	2 mL	24 mL	3 x 24 mL
Buffer RB	1 mL	30 mL	135 mL
DEPC-Treated ddH ₂ O	500 µL	10 mL	30 mL
User Manual	1	1	1

* Add 8 mL (BW-R6618-00) or 96 mL (BW-R6618-01) or 96 mL (BW-R6618-02) 96-100% ethanol to each RNA Wash Buffer bottle before use.

Introduction

The Fungal RNA Miniprep 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 polysachrides and phenolic compounds commonly found in fungal tissues. In combination with RNA 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 Miniprep Kits are ideal for processing multiple fungal samples in parallel in 1 hour. Purified RNA has A260/A280 ratios of 1.8-2.0 and is suitable for RT-PCR, Northern

BW-R6618 Fungal RNA Miniprep Kit

Analysis, Differential display, Poly A+ RNA selection.

The kit combines the reversible binding properties of EZBind RNA technology with a specially designed buffer system which can effectively remove DNA before RNA isolation. The lysate is passed through an EZgene™ DNA Clearance Column which will trap the genomic DNA. And trace genomic DNA can be eliminated by DNase I treatment (See detail in the protocol) when it is necessary.

Storage and Stability

All components can be stored at 4-28 °C . All kit components are guaranteed for 12 months from the date of production.

Before Starting

Prepare all components and get all necessary materials ready by examining this user manual and become familiar with each step and pay special attention to the followings.

Important Notes

- ⊗ Add 2% volume of β-mercaptoethanol to Buffer FLY before use.
- ⊗ Add 8 mL (BW-R6618-00) or 96 mL (BW-R6618-01) or 96 mL (BW-R6618-02) 100% ethanol to each RNA Wash Buffer before use.

Materials not Supplied

- ⊗ Tabletop microcentrifuge .
- ⊗ 100% ethanol
- ⊗ Vacuum manifold if use vacuum protocol.
- ⊗ Nuclease-free microfuge tubes
- ⊗ β-mercaptoethanol
- ⊗ Liquid nitrogen for freezing/disrupting samples
- ⊗ Preheat an aliquot (100 μL per sample) of DEPC-Treated ddH₂O at 65 °C

Working with RNA

Please take a few minutes to read this user manual 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.
- β -mercaptoethanol (β -mercaptoethanol) is key in denaturing endogenous RNases and must be added to an aliquot of Buffer FLY before use. Add 20 μ L of β -mercaptoethanol per 1 ml of Buffer FLY. This mixture can be stored for 1 week at room temperature.

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 -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 fungal tissue cannot be replaced with mechanical homogenizers.

Note: All centrifugation steps must be carried out at room temperature.

1. Weigh **30-100 mg** fungal 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/β-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 clumps are dispersed. RNA cannot be effectively extracted from clumped sample.

Note: Add 20 µL β-mercaptoethanol per 1 ml of Buffer FLY before use. Samples should not be allowed to thaw before Buffer FLY/ β-mercaptoethanol is added.

3. Transfer the cleared lysate to a **DNA Clearance Column** pre-inserted in a **2 mL Collection Tube**. Centrifuge at 13,000 rpm for 2 min. Discard the **DNA Clearance Column** and save the flow-through.

Note: This step is for genomic DNA removal, it is not necessary.

4. Add **0.5 volume absolute ethanol** and mix by vortexing.
5. Apply the entire sample, including any precipitates that may form to a **RNA Column** assembled in a clean **2 mL Collection Tube** (supplied). Close the cap gently. Centrifuge at 10,000 x g for 30 s at room temperature. Discard the flow-through liquid and place the column back into the collection tube.

Optional: on-membrane DNase I digestion: See steps on Page 6.

6. Add **500 µL Buffer RB**, close the tube gently. Centrifuge at 10,000 x g 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**. Close the column gently, Centrifuge at 10,000 x 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 maxi speed to completely dry the membrane.
9. Elution of RNA. Transfer the column to a clean **1.5 mL RNase-free microfuge tube** and elute the RNA with **50-100 µL** of **DEPC-Treated ddH₂O** (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 elutions 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.

Note: It is highly recommended that RNA quality be determined before downstream applications. The quality of RNA can be assessed by denatured agarose gel electrophoresis with the ethidium bromide staining. Several sharp bands should appear on the gel including 28S and 18S ribosomal RNA bands as well as certain populations of mRNA and bands. If these bands smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling or storage, RNA molecule less than 200 bases in length do not efficiently bind to the RNA column. An A260/A280 ratio of 1.8-2.0 corresponds to 90-100% pure nucleic acid.

Options: Removal of genomic DNA using DNase digestion

DNA digestion is necessary for downstream applications that are sensitive to very small amounts of DNA, for example, RT-PCR with low-abundance target. Generally, it is not required to do so since the EZgene RNA purification kit selectively isolates RNA and eliminates most of the DNA. If there is DNA contamination, either reduces the tissue amount or cell .

Catalog#	BW-R6618-00	BW-R6618-01	BW-R6618-02
Preps	4	50	250
DNase I	25 u	260 u	1300 u
1 x DNase I Buffer	300 µL	3 mL	15 mL
DNase Stop Buffer	200 µL	2.4 mL	12 mL

*DNase I , DNase Stop Buffer not supplied. They could be purchased from BEIWO

*Add 800 µL (BW-R6617-00) or 9.6 mL (BW-R6617-01) or 48 mL (BW-R6617-02) 100% ethanol to DNase Stop Buffer before use. The final ethanol is 80% (v/v).

Protocol for Removal of genomic DNA using DNase digestion

1. After loading the sample into the RNA column, proceed to the following step for DNase I digestion.
2. Place column in a clean 2 mL collection tube, and add **500 µL Buffer RB**. Centrifuge as above and discard flow-through. Reuse the collection tube for next step.
3. Add **50 µL DNase I (2U , RNase-free) Mixture** onto the middle of the column and incubate at room temperature for 15 min. 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.

Trouble Shooting Guide

Problem	Possible 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 amount of starting material.
Clogged column	Incomplete disruption or lysis of fungal 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, use Buffer FLY to dissolve the RNA pellet
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 β-mercaptoethanol is added to Buffer FLY.
	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 RNA Wash Buffer has been diluted with 100% ethanol as indicated on bottle. Diluted RNA Wash Buffer must be stored at room temperature. Repeat wash with RNA Wash Buffer.
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 (pH8) to dilute RNA prior to specanalysis.

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us or visit our website.



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