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Biozol RNA Miniprep Kit

(BW-R7311)

Contents

Kit Contents

Catalog #	BW-R7311-00	BW-R7311-01	BW-R7311-02
Preps	10	50	250
RNA Mini Columns	10	50	250
2 mL Collection Tubes	10	50	250
Biozol Reagent	11 mL	55 mL	300 mL
Buffer RB	9 mL	45 mL	225 mL
RNA Wash Buffer*	3 mL	24 mL	3×24 mL
DEPC-treated ddH_2O	2 mL	10 mL	50 mL
Dnase I (2 U/μL, Optional) *	60 μL	300 μL	1500 μL
DNase Stop Buffer (Optional)*	0.5 mL	2.4 mL	12 mL
1.5 mL RNase-free Microfuge Tubes	10	50	250
User Manual	1	1	1

*Add 12 mL (BW-R7311-00) or 96 mL (BW-R7311-01) or 3×96 mL (BW-R7311-02) 100% ethanol to RNA Wash Buffer before use.

*DNase I (Cat#D001) can be purchased from BEIWO separately.

Introduction

EZgene™ Biozol RNA Miniprep Kit provides a rapid and easy method for the isolation of up to 100 μg of total RNA from cultured eukaryotic cells, tissues, bacteria, plant or fungal. The kit allows single or multiple, simultaneous processing of samples in less than 30 min. Normally, up to 1 x 10^6 eukaryotic cells, up to 1 x 10^9 bacterial cells, 100 mg tissue or 100 mg plant samples can be used in a single experiment. While this kit may be used for isolation of RNA from whole blood, we recommend you use the EZgene™ Blood RNA Kit (product # R6411) as it is specifically designed for effective hemolysis and hemoglobin removal and gives higher RNA yields.

RNA purified using the EZgene™ Total RNA method is ready for applications such as RT-PCR, Northern blotting, poly A+ RNA (mRNA) purification, nuclease protection, and *in vitro* translation.

Principle

The EZgene™ Biozol RNA Miniprep Kit uses the reversible binding properties of ezBind matrix, a new silica-based material. By combined the high lysis efficient of Biozol Reagent with OBI

innovative ezBind technology, this kit can extract total cellular RNA from different sources of samples specially for fatty tissues such as brain and adipose tissue. A specifically formulated high salt buffer system allows more than 100 μg of RNA molecules greater than 200 bases to bind to the matrix. Cells or tissues are first homogenized with Biozol Reagent that practically inactivate RNase. After add chloroform, the homogenate is separated into aqueous and organic phase with centrifugation. The aqueous phase which contains RNA then adjusted with ethanol and then applied to the RNA Mini Column to which total RNA binds, while cellular debris and other contaminants are effectively washed away. High quality RNA is finally eluted in DEPC-Treated ddH_2O.

Storage and Stability

Biozol Reagent should be store at 4℃, all other contents should be stored at room temperature (4-28℃). The guaranteed shelf life is 12 months from the date of production when stored as above.

Before Starting

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

- Add 12 mL (BW-R7311-00) or 96 mL (BW-R7311-01) or 3×96 mL (BW-R7311-02) 100% ethanol to RNA Wash Buffer before use.
- **Optional:** Add 800 μL (BW-R7311-00) or 9.6 mL (BW-R7311-01) or 48 mL (BW-R7311-02) 100% ethanol to DNase Stop Buffer before use.
- Whenever working with RNA, always wear latex gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents. During the procedure work carefully but quickly.
- It is very important to determine the correct amount of starting material before the experiment. The maximum amount of starting material is 100 mg. The capacity of the RNA Mini Column is 100 μg. For samples contains high amount of RNA, we suggest to use 30 mg

tissue to start. For samples contains lower level RNA, the maximum amount of starting material (100 mg) can be used.

Materials not Supplied

✓ Chloroform

✓ Isopropanol

Homogenization of Tissues

A. Liquid nitrogen method

➢ Wear gloves and take great care when working with liquid nitrogen. Excise tissue and promptly freeze in a small volume of liquid nitrogen. Grind tissue with a ceramic mortar and pestle under approximately 10 mL of liquid nitrogen and pour the suspension into a pre-cooled 15 mL polypropylene tube. Unless the tube is pre-cooled (in liquid nitrogen), the suspension will boil vigorously possibly causing loss of tissue. When the liquid nitrogen has completely evaporated, add Biozol Reagent and continue with the procedure as outlined below. This is the preferred method of disrupting tissue samples.

B. Rotor-stator homogenizers

➢ Rotor-stator homogenizers effectively homogenize most tissues. The process usually takes less than a minute depending on the tissue. Many Rotor-stator homogenizers operate with differently sized probes or generators that allow processing of small volumes in microfuge tubes.

C. Syringe method

➢ High molecular weight DNA is responsible for the viscosity of cell lysates and can be shredded by passing the sample several times through a narrow needle (19-21 gauges).

Protocol (For spin)

A. Eukaryotic Cells and Tissues

1. Lyse cells or tissues with **1 mL** of **Biozol Reagent**.

   Note: 1mL of Biozol Reagent is sufficient for 10^7 cells or approximately 100 mg disrupted tissue (~30 mm cube).

   For tissue culture cells grown in monolayer (fibroblasts, endothelial cells, etc.),

   Lyse the cells directly in the culture vessel as follows. Aspirate culture medium completely and add Biozol Reagent directly to the cells. Pipette buffer over entire surface of vessel to ensure complete lysis. Transfer lysate to a clean 1.5 mL microfuge tube and proceed to step **2** below. (This method is preferable to trypsinization followed by washing because it minimizes RNA degradation by nuclease contamination.)

   For cells grown in suspension cultures

   Pellet cells at no greater than 1,500 rpm (400 x g) for 5 min. Discard supernatant, add Biozol Reagent, lyse by vortex or pipetting up and down, and transfer to a clean 1.5 mL microfuge tube. Proceed to step **2**.

   For tissue samples

   Determine the size of the samples and homogenize by using one of the methods discussed on page **3**. Unless using liquid nitrogen, homogenize samples directly in Biozol Reagent and proceed to step **2**.

2. Incubate the tube contains homogenate at room temperature for 2 min.

3. Add **0.2 mL** of **chloroform** per **1 mL** of **Biozol Reagent**. Cap sample tubes securely and shake vigorously for 15 s.

4. Centrifuge at 12,000 x g for 3 min at 4℃. The mixture separates into a lower phenol-chloroform phase, an inter phase, and an upper aqueous phase. RNA remains entirely in the aqueous phase.

5. Transfer no more than 80% of the aqueous phase to a fresh tube and add **0.5 volume** of **isopropanol** (96-100%, room temperature). Vortex at maximum speed for 15 s.

6. Apply no more than **700 µL** of the mixture from step **5** onto a **RNA Mini Column**. (Larger volumes can be loaded successively.) A precipitate may form on addition of isopropanol in step **5**. Vortex and add the entire mixture to the column. Centrifuge at 10,000 x g for 30-60 s at room temperature. Discard flow-through and reuse the collection tube.

7. Repeat step **6** by loading the remaining sample to the column. Centrifuge as above and discard flow-through.

8. Place column in a clean **2 mL Collection Tube**, and add **400 µL Buffer RB**. Centrifuge as above and discard flow-through. Reuse the collection tube for step **10**. If on-membrane DNase I digestion is desired, proceed step **9**, otherwise go to step **10**.

9. **Optional: DNase I digestion**

Since RNA resin and spin-column technology actually removes most of DNA without the DNase treatment, it is not necessary to do DNase digestion for most downstream applications. However, certain sensitive RNA applications might require further DNA removal. Following steps provide on-membrane DNase I digestion.

A. For each **RNA Mini Column**, prepare the DNase I digestion reaction mix as follows:

1 x DNase I Buffer	46.5 µL
RNase-free DNase I (2 U/µL)	3 µL
Total volume	50 µL

Note:

- DNase I is very sensitive for physical denaturation, so do not vortex this DNase I mixture. Mix gently by inverting the tube. Prepare the fresh DNase I digestion mixture before RNA isolation.
- DNase I 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 **RNA Mini column**. Make sure to pipet the DNase I digestion mixture directly onto the membrane. DNase I digestion will not be complete if some of the mix stick to the wall or the O-ring of the **RNA Mini Column**.

C. Incubate at room temperature (15-25°C) for 15 min.

D. Add 200 µL DNase Stop Buffer, wait at least 5 min, centrifuge as above and discard flow-through

E. Place column in the collection tube and add **400 µL Buffer RB**. Centrifuge as above and discard flow-through.

10. Place column in the same **2 mL Collection Tube**, and add **600 µL RNA Wash Buffer**. Centrifuge as above and discard flow- through. Reuse the collection tube in step **12**.

11. Wash column with a second **600 µL** of **RNA Wash Buffer** as in step **11**. Centrifuge and discard flow-through.

12. Centrifuge the column for 2 min at full speed (13,000 x g) to completely dry the membrane.

13. Transfer the column to a clean **1.5 mL RNase-free Microfuge Tube** and elute the RNA with **30-50 µL** of **DEPC-treated ddH_2O** (supplied with kit). Make sure to add water directly onto column matrix. Let the column sit at room temperature for 2 min and centrifuge for 1 min at full speed.

    Optional: Reload the eluate into the center of the column for a second elution.

B. EZgene™ Protocol for Bacteria

1. Harvest cells and resuspend in **100 µL TE/lysozyme** and incubate at room temperature for 7 min.

    Note: Centrifuge 10^9 cells at 4,000 x g for 5 min. Discard supernatant and add 100 µL of TE buffer containing lysozyme (0.5 mg/mL for Gram-negative and 4 mg/mL for Gram-positive bacteria). Resuspend cells completely and incubate at room temperature for 7 min.

2. Add **1mL** of **Biozol Reagent** and mix by vortexing for 15 s. Incubate the tube contains homogenate at room temperature for 3 min.

3. Add **0.2 mL** of **chloroform** per **1 mL** of **Biozol Reagent**. Cap sample tubes securely and vortex vigorously for 15 s.

4. Centrifuge at 12,000 x g for 3 min at 4℃, the mixture separates into a lower phenol-chloroform phase, an interphase, and an upper aqueous phase. RNA remains entirely in the aqueous phase.

5. Transfer no more than 80% of the aqueous phase to a fresh tube. Add **0.5 volume** of **isopropanol** (96-100%, room temperature) and vortex at maximum speed for 15 s. A precipitate may form at this point. This will not interfere with RNA purification.

6. Apply no more than **700 µL** of sample from step **5** onto **RNA Mini Column**. (Larger volumes can be loaded successively.) A precipitate may form on addition of ethanol in step **5**. Vortex and add the entire mixture to the column. With the spin Column inside a collection tube, centrifuge at 10,000 x g for 15-30 s at room temperature. Discard flow-through and reuse the collection tube.

7. Repeat step **6** by loading the remaining sample to the column, discard flow-through and collection tube.

8. Proceed as step **8-13** on page **6-7**.

Protocol (For spin/vacuum)

Carry out lysis, homogenization, and loading onto **RNA Mini Column** as indicated previous protocols. Instead of continuing with centrifugation, follow steps blow.

Note: Please read through previous section of this book before using this protocol.

1. Prepare the vacuum manifold according to manufacturer's instruction and connect the **RNA Mini Column** to the manifold.
2. Load the samples from step **5** into **RNA Mini Column**.
3. Switch on vacuum source to draw the sample through the column and turn off the vacuum.
4. **Optional:** Perform on-membrane DNase I digestion steps if sensitive downstream application is desired. (See previous section for details)
5. Wash the column by adding **400 µL Buffer RB,** draw the liquid through the column by turn on the vacuum source.
6. Wash the column by adding **600 µL RNA Wash Buffer,** draw the wash buffer through the column by turn on the vacuum source.
7. Repeat step **6**.
8. Assemble the column into a **2 mL Collection Tube** and transfer the column to a micro centrifuge. Spin at maxi speed for 2 min to dry the column.
9. Place the column in a clean **1.5 mL RNase-free Microfuge Tube** and add **30-50 µL DEPC-treated ddH_2O**. Stand for 1-2 min and centrifuge 1 min to elute RNA.

DNA contamination

Generally **RNA Mini Column** technology will efficiently removes most of the DNA without DNase treatment. However, no RNA extraction procedure can completely remove genomic DNA. For sensitive work (such as RT-PCR or differential display) we suggest that you perform on-membrane DNase I digestion (OBI cat# E1091) or 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. For designing intron-spanning primers, call our technical staff at 400-115-2855 for assistance. We can help design primers suited to your needs.

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 OD unit measured at 260 nm corresponds to 40 μg of RNA per mL. 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 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 absorbance readings of DNA or RNA. However, the EZgene™ Total RNA Kit eliminates the use of phenol and avoids this problem). Store RNA samples at -80℃ in water. Under such conditions RNA prepared with the EZgene™ system is stable for more than a year.

RNA quality

It is highly recommended that RNA quality be determined prior to all analyses. The quality of RNA can best be assessed by denaturing agarose gel electrophoresis and ethidium bromide staining. Two sharp bands should appear on the gel. These are the 28S and 18S (23S and 16S for bacteria) ribosomal RNA bands. If these bands smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling or storage. Although RNA molecules less than 200 bases in length do not efficiently bind the ezBind matrix, a third RNA band, the tRNA band, may be visible when a large number of cells are used.

Trouble Shooting Guide

Problem	Possible reason	Suggested Improvement
Little or no RNA eluted	RNA remains on the column	● Repeat elution. ● Preheat DEPC-Treated ddH_2O to 65℃ prior to elution. ● Incubate column for 10 min with water prior to centrifugation.
	Column is overloaded	Reduce quantity of starting material.
Clogged column	Incomplete homogenization	● Completely homogenize sample. ● Increase centrifugation time. ● Reduce amount of starting material
Degraded RNA	Source	● Freeze starting material quickly in liquid nitrogen. ● Do not store tissue culture cells prior to extraction unless they are lysed first. ● Follow protocol closely, and work quickly.
	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 4 volumes of 100% ethanol as indicated on bottle. ● RNA Wash Buffer must be stored and used at room temperature. ● Repeat wash with RNA Wash Buffer.
DNA contamination	Draw into the middle phase when draw the supernatant.	Digest with RNase-free DNase and inactivate at 75℃ for 5 min.

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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