Ver: 2008

Biozol Reagent

（BW-R1020）

Contents

Kit Contents

Catalog #	BW-R1020-00	BW-R1020-01	BW-R1020-02
Biozol Reagent	5 mL	100 mL	5 x100 mL
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Notes： Biozol Reagent contains Guanidine isothiocyanate and phenol, please be careful in your experiment.

Introduction

Biozol Reagent is designed to isolate high quality total RNA from cultured eukaryotic cells, tissues, plant, fungal, or bacterial within 30 minutes. Normally, up to 1×10^6 eukaryotic cells, 1 x 10^9bacterial cells and 100 mg tissue or 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 Miniprep Kit (Catalog#R6411) as it is specially designed for effective hemolysis and hemoglobin removal and gives higher RNA yields.

Isolated RNA can be used in RT-PCR, Northern blotting, mRNA purification, *in vitro* translation and other downstream experiments.

Principle

Components in the Biozol Reagent would inhibited RNase activity after cells or tissues lysed by Biozol Reagent. When chloroform was added, the lysate was divided into aqueous phase and organic phase. RNA exists in the aqueous phase. After treating the aqueous with isopropanol, washing with 75% ethanol, drying at room temperature or vacuum drying, high quality RNA can be obtained when dissolving with DEPC-treated ddH_2O.

Stable and Stability

Biozol Reagent should be stored at 4℃, the guaranteed shelf life is 12 months from the date of production.

Before Starting

Please take a few minutes to read this manual thoroughly and become familiar with the protocol.

Prepare all materials required before starting to minimize the RNA degradation.

- Whenever working with RNA, always wear disposable gloves to minimize RNase contamination, use disposable RNase-free pipettes, pipette tips and tubes 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 experiment, the maximum dosage is 100 mg, 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

- Isopropanol
- Chloroform
- Ethanol

Homogenization of tissues

A. Liquid nitrogen method

Immediately freezes the sample in the liquid nitrogen and grinds the tissue into fine powder under liquid nitrogen. Pour the suspended powder into the pre-cooled centrifuge tube, unless the tube is pre-cooled (in liquid nitrogen), the suspension will boil vigorously causing loss of sample. When the liquid nitrogen completely evaporated, add Biozol Reagent before sample melting.

B. Rotor-stator homogenizers

Rotor-stator homogenizers can efficiently and conveniently homogenize most tissues. Many rotor-stator homogenizers operate with different sized probes or generators that allow processing of small volumes in microfuge tubes.

C. Syringe method

High molecular weight DNA increases the viscosity of the lysate, it can be shredded by passing the sample several times through a narrow needle (19-21 gauges) .

Protocol

A：Eukaryotic cells and tissues

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

   Note:1 mL of Biozol Reagent is sufficient for 10^7cells or 100 mg disrupted tissue.

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

   Remove growth media and add**1 mL**of**Biozol Reagent** directly to the culture dish to lyse the cells. Pipet the lysate up and down several times to homogenize. Transfer lysate to a clean 1.5 mL RNase-free microfuge tube.

   For cells grown in suspension

   Pellet the cells and centrifuge at no more than 1,500 rpm (400 x g) for 5 min, discard the supernatant, add**1 mL**of**Biozol Reagent**, pipet the lysate up and down several times to homogenize, transfer to a clean 1.5 mL RNase-free microfuge tube.

   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 **1 mL**of**Biozol Reagent** and proceed to step **2**. For special tissue samples such as liver, spleen, bone and cartilage, the amount of **Biozol Reagent** needs to be increased or reduced.

2. Incubate at room temperature for 2 min.
3. Add**0.2 mL** of **chloroform** per **1 mL** of **Biozol Reagent**. Securely cap the tube 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, 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 new tube and add **0.5 volume** of **isopropanol** (96-100%, room temperature). Vortex at maximum speed for 15 s.
6. Centrifuge at 12,000 xg for 10 min at 4℃, the bottom of the tube will precipitate after centrifugation, discard the supernatant.
7. Resuspend the pellet in**1 mL75% ethanol** (to prevent the dispersal of ethanol)by following the tube wall gently, wash the precipitation by gently shaking the centrifuge tube. Centrifuge

at 7,500 x g for 5 min, discard the supernatant, and try to remove residual ethanol, so as to reduce salt ion pollution.

8. Repeat step **7**.
9. Vacuum or air dry the RNA pellet for 5-10 min.Add **30-50 µL** DEPC-treated ddH_2O to dissolve RNA, storeat -80℃.

B：Bacterial

1. Harvest cells and resuspend in **100 µL TE/lysozyme,** 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 atmaximum speed for 15 s. A precipitate may form at this point, this will notinterfere with RNA purification.
6. Centrifuge at 12,000 xg for 10 min at 4℃, the bottom of the tube precipitates after centrifugation.
7. Follow the steps **7-9**onpage**4-5**.

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°C 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 RNAeluted	Special tissue sample, extremely differentiated, or with few cells.	● Repeat elution. ● Preheat DEPC-Treated ddH_2O to 65℃ prior to elution. ● Preheat the column to 65℃for 10 min beforecentrifugation. ● Sample was not fresh, RNA was degraded.
	RNA was lost during the operation.	● Experiment as quickly and carefully as possible. ● Use RNase-free tubes and tips.
Too much impurities	Incompletelysis.	● Completely homogenize sample. ● Reduce amount of starting material. ● Do not draw into the middle phase when draw the supernatant.
RNA degraded	Sample source	● Freeze starting material quickly in liquid nitrogen. ● Use fresh sample and lysis quickly. ● Follow the protocol closely, operate quickly.
	RNasecontamination	● Ensure not to introduce RNase during the procedure. ● Check buffers for RNase contamination.
Problem indownstreamapplications	Salt carry-overduring elution	● Wash again with 75% ethanol. ● Remove residual ethanol after washing with 75% ethanol each time. ● Use 75% ethanol to wash the wall of centrifuge tube.
DNA contamination	Draw into the middle phase when draw the supernatant.	● Digest with RNase-free DNase I and incubate 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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