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Tissue RNA Miniprep Kit

（BW-R6311）

Contents

Kit Contents

Catalog#	BW-R6311 -00	BW-R6311 -01	BW-R6311 -02
Preps	10	50	250
DNA Clearance Columns	10	50	250
RNA Mini Columns	10	50	250
2 mL Collection Tubes	20	100	500
Buffer LY	6 mL	28 mL	135 mL
Buffer RB	6 mL	30 mL	135 mL
RNA Wash Buffer*	3 mL	24 mL	3 x 24 mL
DEPC-treated ddH_2O	1 mL	10 mL	30 mL
1.5 mL RNase-free Microfuge Tubes	10	50	250
User Manual	1	1	1

Introduction

The EZgene™ Tissue RNA Miniprep Kit provides an easy and fast method for isolating total RNA from tissues, cultured cells within 30 min. Only trace genomic DNA exists in the purified RNA. This kit purifies up to 100 µg of total RNA from eukaryotic cells or animal tissues. The purified RNA is ready for RT-PCR, Northern blotting, polyA+ RNA purification, nuclease protection, and *in vitro* translation.

The kit combines the reversible binding properties of ezBind RNA technology with a specially designed buffer which effectively removes genomic DNA before RNA isolation. The trace genomic DNA can be eliminated by DNase I treatment (See detail in the protocol) when it is necessary.

Storage and Stability

The guaranteed shelf life is 12 months from the date of production. All components can be stored at room temperature (4-28℃).

Before Starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each step.

Important

☼ Determine the volume of Buffer LY to be used and add 20 μL of β-mercaptoethanol (β-Me) per 1 mL Buffer LY before use. Buffer LY contains β-Me can be stored at room temperature for up to 1 month.

☼ Crystals may form in Buffer LY, dissolve the precipitates at 37℃ before use.

☼ Add 12 mL (BW-R6311-00) or 96 mL (BW-R6311-01) or 96 mL (BW-R6311-02) 96-100% ethanol to each RNA Wash Buffer bottle before use. The final ethanol is 80% (v/v).

Materials not Supplied

✪Tabletop microcentrifuge.

✪Vacuum manifold if use vacuum protocol.

✪β-mercaptoethanol.

✪96-100% ethanol.

Note: *Perform all steps including centrifugation at room temperature.*
Carry out the procedure as quickly as possible to reduce the RNA degradation.

Safety Information

Buffer LY contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste, wear gloves and protective eyewear when handling.

Disruption and homogenization of tissue samples

It is critical to disrupt and homogenize the samples completely and properly for high quality RNA yield. The purpose for homogenization is to reduce the viscosity by shearing genomic DNA and other high molecular weight cell components to create a homogenous lysate. Incomplete homogenization may result in clogging the column and reducing the RNA yield.

1. Sample disruption by mortar and pestle

- Excise tissues and freeze in liquid nitrogen immediate.
- Grind the sample with ceramic mortar and pestle to a fine powder under liquid nitrogen.
- Transfer the suspension into a tube precooled in liquid nitrogen and allow the liquid nitrogen to evaporate while the samples remain frozen.
- Add Buffer LY before the sample gets thawed.

2. Homogenization using homogenization columns

It is a fast and efficient way to homogenize the samples using BEIWO's homogenization column. Up to 700 μL of samples can be loaded per column. Homogenization columns can be purchased from BEIWO (Cat#R1800) separately.

3. Rotor-Stator for sample disruption and homogenization

Using a proper size probes and generator, the process simultaneously disrupts and homogenizes most of samples.

4. Bead milling for sample disruption and homogenization

Cells and tissues can be disrupted and homogenized by rapid agitation in the presence of glass beads in Buffer LY. Use 4-8 mm glass beads for animal tissues, 0.5 mm for yeast cells and 0.1 mm for bacterial samples.

Determine amounts of samples to be processed

The yield depends on the tissue and cells to be processed. Please refer to Table 1 to determine the amount of sample and expected yield.

Table 1. Typical yield of total RNA per column

Sample	10 mg/500 µL Buffer HLY	Total RNA Yield (µg)
Liver	10 mg	50 (10 mg tissue)
Kidney	10 mg	20-30 (10 mg tissue)
Muscle*	10 mg	20 (10 mg tissue)
Spleen	10 mg	30-40 (10 mg tissue)
Heart*	10 mg	50 (10 mg tissue)
Brain**	10 mg	80 (10 mg tissue)
Lung	10 mg	10-20 (10 mg tissue)
Pancreas	10 mg	20 (10 mg tissue)
HeLa Cells	$1x10^6$	15 ($1x10^6$ cells)
293HEK	$1x10^6$	12 ($1x10^6$ cells)
COS-7	$1 x10^6$	30 ($1x10^6$ cells)
NIH/3T3	$1x10^6$	10 ($1x10^6$ cells)

***Note:**It is normally difficult to isolate RNA from heart, muscle, and skin tissue using the regular RNA isolation procedure due to the rich contents of connective tissue, collagen, and contractile proteins. Optimization with the addition of proteinase K digestion that enables the removal of proteins described above is needed. We suggest you use **Biozol RNA Purification Kit (Cat#BW-R7311) for heart, muscle, and skin tissue.**

****Note:**For isolating from lipid rich animal tissue, such as thymus and brain tissue, we recommend you use **Biozol RNA Purification Kit (Cat#BW-R7311) for lipid-rich animal tissue.**

Protocol (For extracting total RNA from cultured cells)

1. Cell preparations: (Do not use more than $5x10^6$ of cells).

- **Suspension cultured cells:** determine the cell numbers and collect cells by centrifuging at 300 x g for 5 min. Remove all supernatant completely by aspiration and proceed quickly to step **2**. Work as quick as possible to reduce RNA degradation.
- **Adherent cultured cells:** Determine cell numbers and aspirate the medium completely with a pasteur pipet. Go to step **2** immediately by adding Buffer LY.

  Note: Supernatant must be removed completely. Residual supernatant may inhibit cell lysis and thus affect the RNA yield.

2. **Suspension cells:** Flicking the tube to loosen the cell pellet and add **500 µL Buffer LY.**

   Adherent cells: Add **500 µL Buffer LY** directly into the dish. Use pipet tip to mix and transfer the cell lysate to a 1.5 mL tube.

   Note: Determine the volume of Buffer LY to be used and add 20 µL of β-mercaptoethanol (β-ME) per 1 mL Buffer LY before use. Buffer LY contains β-ME can be stored at room temperature for up to 1 month.

3. Homogenize the lysate by vortexing vigorously or repeated pipetting until the sample is uniformly homogenized.

4. Transfer all the lysate to a **DNA Clearance Column**. Centrifuge at 12,000 rpm for 2 min. Discard the **DNA Clearance Column** and save the flow-through.

   Note: This step is for genomic DNA removal.

5. Add **1/2 volume 100% ethanol** into the lysate (for example: 250 µL 100% ethanol for 500 µL lysate) and pipet 5 times to mix the solution. Vortex briefly if any precipitations.

6. Transfer the solution to a **RNA Mini Column** and centrifuge at 12,000 rpm for 1 min. Discard the collection tube with the flow-through and put the column into a new **2 mL Collection Tube**.

7. Add **500 µL Buffer RB** to the column and centrifuge at 12,000 rpm for 30 s. Discard the flow-through.

8. Add **500 µL RNA Wash Buffer** *(Add ethanol before use)* to the column and centrifuge at 12,000 rpm for 30 s. Discard the flow-through.

9. **Optional:** Add another **500 µL RNA Wash Buffer** to the column and centrifuge at 12,000 rpm for 30 s. Discard the flow-through and collection tube, put the column, with the lid open, into a new **2 mL collection tube**. Centrifuge the column at 12,000 rpm for 1 min.

   Note: The residual ethanol will be removed more efficiently with the lid of the column open.

10. Transfer the column to a **1.5 mL RNase-free Microfuge Tube** and add **50-100 µL DEPC-treated ddH_2O** to the center of the column. Centrifuge at 12,000 rpm for 1 min to elute the RNA. Store the RNA solution at -20℃.

Protocol (For extracting total RNA from animal tissues)

1. Quickly weight an appropriate mass tissue according to Table 1 (Page **5**) and immediately transfer the tissue into a 1.5 mL tube containing **500 µL Buffer LY** (*add β-mercaptoethanol before use*) and homogenize the tissue by a rotor starter or ultrasonic homogenizer on ice.

   Note: Determine the volume of Buffer LY to be used and add 20 µL of β-mercaptoethanol (β-ME) per 1 mL Buffer LY before use. Buffer LY contains (β-ME) can be stored at room temperature for up to 1 month.

   Note: Do not use over 30 mg of tissue per column as this causes incomplete tissue digestion and genomic DNA contamination.

2. Transfer the cleared lysate to a **DNA Clearance Column** pre-inserted in a **2 mL Collection Tube**. Centrifuge at 12,000 rpm for 2 min. Discard the **DNA Clearance Column** and save the flow-through.

   Note: This step is for genomic DNA removal.

3. Add **1/2 volume** of the **100% ethanol** to the lysate (for example: 250 µL 100% ethanol for 500 µL lysate).

4. Transfer the solution into a **RNA Mini Column** and centrifuge at 12,000 rpm for 1 min. Discard the flow-through liquid and collection tube, put the column into a new **2 mL Collection Tube**.

5. Add **500 µL Buffer RB** to the column and centrifuge at 12,000 rpm for 30 s. Discard the flow-through liquid and put the column back to the collection tube.

6. Add **500 µL RNA Wash Buffer** to the column and centrifuge at 12,000 rpm for 1 minute. Discard the flow-through.

7. **Optional:** Add **500 µL RNA Wash Buffer** to the column and centrifuge at 12,000 rpm for 30 s. Discard the flow-through and collection tube, put the column, *with the lid open*, into a new **2 mL Collection Tube**.

8. Centrifuge the column at 12,000 rpm 2 min. It is critical to remove residue ethanol for optimal elution in the following step.

9. Place the column to a **1.5 mL RNase-free Microfuge Tube** and add **50-100 µL DEPC-treated**

ddH$_2$O to the column and centrifuge at 12,000 rpm for 1 min. The RNA is in the flow-through liquid. Store the RNA solution at -20℃.

Options: Removal of genomic DNA using DNase I 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#	**D001-00**	**D001-01**	**D001-02**
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 (Cat#D001) can be purchased from BEIWO separately.

*Add 800 µL(D001-00) or 9.6 mL (D001-01) or 48 mL (D001-02) 100% ethanol to DNase Stop Buffer before use. The final ethanol is 80% (v/v).

DNase I digestion protocol

1. After loading the sample into the **RNA Mini 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 12,000 rpm for 1 min. Discard the flow-through. Add **300 µL RNA Wash Buffer** to the column and centrifuge at 12,000 rpm for 1 min. Discard the flow-through.

Trouble Shooting Guide

Problems	Possible reasons	Suggested Improvements
Low A_{260}/A_{280} ratios	Protein contamination	Do Phenol:Chloroform extraction. Loss of total RNA (up to 40%) should be expected.
Low A_{260}/A_{280} ratios	Guanidine Thiocyanate contamination	Add 2.5 volumes of ethanol and 0.1 M NaCl (final concentration) to precipitate RNA. Incubate for 30 min at -20℃. Centrifuge at 10,000 × g for 15 min at 4℃. Resuspend the RNA pellet in DEPC-treated ddH_2O.
Low Yield	RNA in sample degraded	Freeze samples immediately in liquid nitrogen and store at -70℃ after collect it.
Low Yield	The binding capacity of the membrane in the spin column was exceeded	Use of too much tissue sample exceeding the binding capacity of spin column will cause the decreasing of total RNA yield.
Low Yield	Ethanol not added to buffer	Add ethanol to the RNA Wash Buffer and DNase Stop Buffer before purification.
Genomic DNA contamination	Too much total RNA sample was used in RT-PCR.	Reduce total RNA amount used in RT-PCR to 50-100 ng.
Genomic DNA contamination	The sample may contain too much genomic DNA.	Reduce the amount of starting tissue in the preparation of the homogenate. Most tissues will not show a genomic DNA contamination problem at 30 mg or less per prep. Reduce cell numbers to 1-2 x 10^5 or increase buffer volume and do multiple loading to column.

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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