Ver: 2211

Mollusk RNA Miniprep Kit

（BW-R6619）

Contents

Kit Contents

Catalog#	BW-R6619-00	BW-R6619-01	BW-R6619-02
Preps	4	50	250
DNA Column	4	50	250
RNA Columns	4	50	250
Collection Tubes	8	100	500
1.5 mL RNase-free microfuge tube	4	50	250
Buffer BML	2 ml	20 mL	100 mL
Buffer LY	4 mL	30 mL	130 mL
Buffer RB	4 mL	45 mL	220 mL
RNA Wash Buffer *	2 mL	24 mL	3 x24 mL
DEPC-Treated ddH_2O	500 µL	20 mL	70 mL
User Manual	1	1	1

* Add 8 mL (BW-R6619-00) or 96 mL (BW-R6619-01) or 96 mL (BW-R6619-02) 96-100% ethanol to each RNA Wash Buffer bottle before use.

Introduction

Mollusks RNA Miniprep Kit is designed for efficient recovery of total RNA greater than 200 nt from molluscs, roundworms, flatworms, and other invertebrate tissue samples rich in mucopolysaccharides. The procedure relies on the well established properties of the cationic detergent, cetyltrimethyl ammonium bromide (CTAB), in conjunction with the selective RNA binding of silica membrane. Samples are homogenized and lysed in a high salt buffer containing CTAB and extracted with chloroform to remove mucopolysaccharides and denature proteins. Following a rapid alcohol precipitation step, binding conditions are adjusted and RNA further purified using ezBind RNA spin columns. In this way salts, proteins and other contaminants are removed to yield high quality total RNA suitable for downstream applications such as reverse transcription, poly(A)+ mRNA selection, and hybridization techniques.

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 a EZgeneTM 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℃. 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

✪Determine amount of Buffer BML and Buffer LY to be used, add 20 μL β- mercaptoethanol (β-ME) per 1 mL Buffer BML or LY. Buffer BML and Buffer LY contains β-ME can be stored at room temperature for up to 1 month. β-ME is key in denaturing endogenous RNase.

✪Add 8 mL(BW-R6619-00) or 96 mL (BW-R6619-01) or 96 mL (BW-R6619-02) 100% ethanol to each RNA Wash Buffer before use.

✪During shipment or storage under room temperature, crystal may form in certain buffers. Dissolve the precipitates at 37℃ before use.

✪Do not freeze the buffers at any time.

✪Preheat the Buffer RB and DEPC-treated ddH2O (100 μL per sample) at 65°C.

✪Smaples: fresh tissue is preferred for RNA integrity.

Materials not Supplied

✪Tabletop microcentrifuge .

✪100% ethanol

✪β-mercaptoethanol for denaturing the endogenous RNase.

✪Chloroform and isoamyl alcohol (24:1) for removal of polysaccharides and proteins.

Avoiding RNase Contamination

Please prepare materials as following instructions when working with RNA.

1. For RNA use only: Keep a separate set of pipettors for RNA use to avoid contamination with RNases. Avoid touching the barrel or metal ejector to the sides of tubes.
2. **Solutions:** Store solutions in small aliquots and discard each aliquot after use.
3. **Electrophoresis apparatus:** Wash with detergent solution, rinse in H2O, and dry with ethanol. Then fill with 3% solution of H2O2 (Don't use DEPC solutions because it will break down the plastic), incubate 10 mins at room temperature and rinse with DEPC treated H2O.
4. **Glassware:** Bake glassware at 300° C for 4 hours or 180° C or higher for several hours. Alternatively, soak glassware in freshly prepared 0.1% (v/v) DEPC in water or ethanol for 1 hour, drain, and autoclave (I t is necessary to destroy any unreacted DEPC which can otherwise react with other proteins and RNA).
5. **Plasticware:** Treat plasticware with DEPC. Use RNase-free disposable tips and tubes. Use sterile forceps to transfer items to racks.
6. **Gloves:** Use gloves from a fresh box at all times. Don't touch the gloves to any surface that might be contaminated with RNases.
7. **Work carefully and quickly during the procedure.**

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.

Stabilization of RNA in harvested animal tissues

The intact of RNA in harvested tissue will be protected with the addition of RNA Secure solution (Not Suppied).

Cut the tissue into slices less than 0.5cm thick and immediately add at least 15 volumes of RNAsecure solution, for example, 150 uL RNAsecure solution per 10 mg tissue.

Store at room temperature for up to 24 hours, at 4°C for up to a week, and – 20°C or –70°C for long term.

RNA quality

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.

Protocol for Total RNA Extraction From Mollusks (Invertebrates)

1. Grind no more than 30 mg tissue in liquid nitrogen with mortar and pestle and transfer the powder into a clean 1.5 ml microcentrifuge tube.

   Note: The procedure could be scaled up with the increase of starting material and volumes of all buffers. With RNA binding capacity of 100 μg, tissue more than 50 mg is not suggested. Less tissue or more buffer is suggested for hard -to-lysis tissue.

I. Quick protocol: follow step 2 to 4, and proceed to step 10.

2. Add **350 μL Buffer LY** (*Add **β-ME** before use)* and vortex vigorously to make sure that all clumps are dispersed.

   Note: Complete dispersion is critical for RNA extraction.

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. Carefully transfer the flow-through to a clean 1.5 ml microfuge tube. Avoid the milky interface containing contaminants and inhibitors.

5. Add **350 μL 100% ethanol** and mix by sharp hand shaking.

II. Suggested but not mandatory protocol: follow step 5 to 9, and proceed to step 10.

6. Add **350 μL Buffer BML** *(Add **β-ME** before use)* and vortex vigorously to make sure that all clumps are dispersed.

   Note: Complete dispersion is critical for RNA extraction.

7. Add **350 μL chloroform:isoamyl alcohol** (24:1) and vortex to mix. Centrifuge 10,000 x g for 2 min at room temperature. Carefully transfer the supernatant to a clean 1.5 ml microfuge tube. Avoid the milky interface containing contaminants and inhibitors.

   Note: This step will remove much of the polysaccharides and proteins from solution and improve the downstream application.

8. Add **1 volume** of **isopropanol** and mix to precipitate RNA. Immediately centrifuge 10,000 x g for 2 min at room temperature. Remove the supernatant completed *(Avoiding disturbing RNA pellet)*. Invert the microfuge tube on a paper towel for 1 min to allow residual liquid to drain.

9. Add **100 μL** of **Buffer LY** *(Add **β-ME** before use, and pre-heated to 65°C)* and vortex to

resuspend the pellet.

Note: Pre-heating the buffer to 65°C is necessary to effectively dissolve the RNA.

10. Add **350 µL Buffer LY** (*Add **β-ME** before use)* and **250 µL 100% ethanol** and mix by sharp hand shaking.
11. Immediately apply the entire mixture (including any precipitation) to the RNA column with collection tube. Centrifuge 10,000 x g for 15 sec at room temperature to let the solution pass through the column. Discard the flow-through liquid and reinsert the column into the collection tube.
12. Add **500 µL Buffer RB** and centrifuge at 10,000 x g for 15 sec. Discard the flow-through liquid and collecting tube.
13. Place the column in a clean 2 mL collection tube, and add **500 µL RNA Wash Buffer** *(Add ethanol before use)*. Centrifuge at 10,000 x g for 15 sec at room temperature and discard flow-through and reinsert the column into the collection tube. Repeat this step.
14. Centrifuge the DNA column for **2 min at full speed** to completely dry the ethanol remain on the membrane.

    Note: Complete removal of residual ethanol is critical for RNA elution.
15. Transfer the column to a clean 1.5 ml microfuge tube. Add **50-100 µL** of **DEPC-Treated ddH_2O** to the center of the membrane to elute the RNA. Centrifuge 1 min at maximum speed. 80% RNA is recovered with first elution.
16. **Optional:** A second elution will increase the yield while lower the concentration.

    Note: For higher RNA concentration, reload the first eluate for the second elution.
17. Determine the concentration and purity of RNA, measure absorbance at 260 nm and 280 nm in a spectrophotometer. A ratio of 1.8-2.0 corresponds to 90%-100% pure nucleic acid. Store RNA samples at - 70°C in water.

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-R6619-00**	**BW-R6619-01**	**BW-R6619-02**
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-R6619-00 ） or 9.6 mL (BW-R6619-01) or 48 mL (BW-R6619-02) 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 reason	Suggested Improvement
Low A_{260}/A_{280} ratios	Protein contamination	Do a 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 0.1M NaCl (final concentration) to precipitate RNA. Incubate for 30 min at -20°C. Centrifuge at 10,000 g for 15 min at 4°C. Resuspend the RNA pellet in DEPC-treated water.
Low Yield	RNA in sample degraded	Freeze samples immediately in liquid nitrogen and store at -70°C 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 1-2x10^6 or increase buffer volume and do multiple loadings 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.

Contact Us: 400-115-2855

www.beiwobiomedical.com

Customer Support:

market@beiwobiomedical.com

Technical Support:

tech@beiwobiomedical.com