Ver: 2211

Bacterial RNA Miniprep Kit

（BW-R6616）

Contents

Kit Contents

Catalog#	BW-R6616-00	BW-R6616-01	BW-R6616-02
Preps	4	50	250
Buffer LY	2.4 mL	28 mL	135 mL
Buffer RB	3 mL	30 mL	135 mL
RNA Wash Buffer	2 mL	24 mL	3 x 24 mL
DEPC-Treated ddH_2O	500 µL	10 mL	30 mL
RNA Columns	4	50	250
DNA Clearance Column	4	50	250
Collection Tubes	8	100	500
1.5 mL RNase-free microfuge tube	4	50	250
Lysozyme	1.2 mg	15 mg	75 mg
User Manual	1	1	1

* Add 8 mL (BW-R6616-00) or 96 mL (BW-R6616-01) or 96 mL (BW-R6616-02) 96-100% ethanol to each RNA Wash Buffer bottle before use.

Introduction

The Bacterial RNA Miniprep Kit provides an easy and fast method for isolating total RNA from Gram-positive (B. subtilis) Or Gram-negative (E. coli) Bacteria within 30 min. 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 kit components are guaranteed for 12 months from the date of production. Reconstituted Lysozyme store at -20°C. All other materials at 4-28°C.

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 1% volume of β-mercaptoethanol to Buffer LY before use and store at 4°C.

✪Add 8 mL(BW-R6616-00) or 96 mL (BW-R6616-01) or 96 mL (BW-R6616-02) 100% ethanol to each RNA Wash Buffer before use.

✪Prepare a lysozyme stock solution at 3 mg/mL or 0.4 mg/mL with Elution Buffer or TE Buffer and aliquot into adequate portions. Store each aliquot at -20 ℃ and thaw before use.

Materials not Supplied

✪Tabletop microcentrifuge .

✪100% ethanol

✪Vacuum manifold if use vacuum protocol.

Note: Perform all steps including centrifugation at room temperature

Protocol For Extracting Total RNA From Gram- positive (B. subtilis) Or Gram-negative (E. coli) Bacteria

1. Grow an overnight bacterial culture in the appropriate media at an appropriate temperature. In the following day, dilute the culture 1:50 with media and grow until the OD600 reads at 0.6-1.0. This should only take a few hours.

   If the growth is too slow, reduce the dilution factor. Do not use the overnight culture for RNA isolation!
2. Harvest no more than 3 mL culture (< $5x10^8$) by centrifugation at 3,000 rpm for 10 min.
3. Carefully remove the supernatant as much as possible.
4. Resuspend the pellet in ***100 µL*** freshly prepared ***TE Buffer(10mM,Tris- HCL,PH 8.0;1mM EDTA,PH 8.0) or Elution Buffer (10mM Tris-HCL, PH 8.5)*** containing lysozyme. (***Use 3 mg lysozyme per 1 mL TE Buffer for Gram-positive bacteria and 0.4 mg/mL lysozyme for Gram-negative bacteria***). Mix by tapping gently.
5. Incubate the resuspended pellet at room temperature for 5-10 min for Gram-positive bacteria, or 3-5 min for Gram-negative bacteria.
6. Add ***400 µL Buffer LY*** . Mix gently. 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.
7. Transfer flow-through to a new RNase-free tube. Add ***0.5 volume 100% ethanol*** to the lysate (For example: 250 µL 100% ethanol for 500 µL lysate).

   Ensure that β-mercaptoethanol has been added before use
8. Transfer the solution into the binding column and centrifuge at 13,000 rpm for 1 min. Discard the collection tube with the flow-through and put the column back to a new collection tube.
9. Add **500 µL Buffer RB** to the column and centrifuge at 13,000 rpm for 30s. Discard the flow-through.
10. Add another ***500 µL RNA Wash Buffer*** to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through.

    Ensure that ethanol has been added to RNA Wash Buffer before use.
11. Add another ***500 µL RNA Wash Buffer*** to the column and centrifuge at 13,000 rpm for 30 s. Discard the flow-through and collection tube, put the column into a new collection tube.

12. Centrifuge the column at 13,000 rpm, with the lid open, for another 1 min. It is critical to remove residual ethanol for optimal elution.

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13. Place the column to a RNase-free 1.5 mL tube, add ***50-100 μL DEPC- treated ddH_2O*** to the column and centrifuge at 13,000 rpm for 2 min. The RNA is in the flow-through liquid. Store the RNA solution at -20 ℃.

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-R6616-00**	**BW-R6616-01**	**BW-R6616-02**
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-R6616-00 ） or 9.6 mL (BW-R6616-01) or 48 mL (BW-R6616-02) 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.

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