Ver: 2211

Plant RNA Miniprep Kit

（BW-R6611）

Contents

Kit Contents

Catalog#	BW-R6611-00	BW-R6611-01	BW-R6611-02
Preps	4	50	250
Buffer RLY	4 mL	55 mL	275 mL
Buffer RB	2 mL	40 mL	200 mL
RNA Wash Buffer	2 mL	24 mL	3 x 24 mL
DEPC-Treated H2O	3 mL	10 mL	50 mL
Plantaid	400 µL	5.5 mL	27.5 mL
RNA Columns	4	50	250
DNA Clearance Column	4	50	250
2 mL Collection Tubes	8	100	500
1.5mLRNase-free microfuge tube	4	50	250
User Manual	1	1	1

* Add 8 mL (BW-R6611-00) or 96 mL (BW-R6611-01) or 96 mL (BW-R6611-02) 96-100% ethanol to each RNA Wash Buffer bottle before use.

Introduction

The Plant RNA Miniprep Kit provides an easy and fast method for isolating RNA from difficult plant tissues 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 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

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

✪Add 8 mL(BW-R6611-00) or 96 mL (BW-R6611-01) or 96 mL (BW-R6611-02) 100% ethanol to each RNA Wash Buffer before use.

Materials not Supplied

✪Tabletop microcentrifuge .

✪100% ethanol

Note: Perform all steps including centrifugation at room temperature

Protocol for Total RNA Extraction From Plant Tissue

1. Weigh **100 mg** plant tissue in a 2 mL tube. Freeze the plant tissue in liquid nitrogen and grind using a rotor starter.
2. Transfer **10 volume (1 mL) Buffer RLY/β-mercaptoethanol** and **1 volume (100μL) Plantaid** to the tube containing the plant tissue immediately. Grind using a rotor starter again. Spin at 12000 rpm for 2 min.

   Ensure that β--mercaptoethanol has been added before use. 10 μL β- mercaptoethanol should be added in 1 mL RLY.
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.
4. Add **0.5 volume 100% ethanol** to the lysate (for example: **250 μL 100% ethanol** for **500 μL lysate**).
5. Transfer the solution into a RNA column and centrifuge at 13,000 rpm for 1 min. Discard the collection tube with the flow-through and put the column back to the collection tube.
6. Add **500 μL Buffer RB** to the column and centrifuge at 13,000 rpm for 30s. Discard the flow-through.
7. Add **500 μL RNA Wash Buffer** to the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through.

   Ensure that ethanol has been added to RNA Wash Buffer before use.
8. 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, **with the lid open**, back to the collection tube.
9. Centrifuge at 13,000 rpm for 2 min. Discard the flow-through.

   NOTE：The residual ethanol will be removed more efficiently with the column lid open during centrifugation.
10. Place the column to a RNase-free 1.5 mL tube, add **30-50 μL DEPC-treated ddH2O** to the column and centrifuge at 13,000 rpm for 1 min. The RNA is in the flow-through liquid. Store the RNA solution at -20°C. Reload the eluted RNA solution to the column and centrifuge at 13,000 rpm again.

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 A_{260}/A_{280} ratio of 1.8-2.0 corresponds to 90-100% pure nucleic acid.

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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