EasyPrep™ Plant Total RNA Extraction Miniprep Manual

Catalog# R03-01, R03-02

For research use only. Not intended for diagnostic testing.

(December 2023)

Contents

Introduction

The EasyPrep™ plant total RNA kit provides an easy and fast method for isolating total RNA from plant tissues within 30 min. The kit is especially efficient on purifying RNA from plants that contain high levels of polysaccharides, phenolic compounds, polyphenolic compounds and/or tannins. Furthermore, the kit can also be used for purifying RNA from animal cells. A separate protocol for animal cell is included.

Only trace genomic DNA exists in the purified RNA, which can be eliminated by DNase I treatment when it is necessary.

Storage and Stability

All components can be stored at room temperature. The kit is guaranteed for 1 year from the date of purchasing.

Kit Contents

Catalog#	**R03-01**	**R03-02**
Preps	50	250
RNA Columns	50	250
Homogenization Column	50	250
Buffer PLY	26 mL	130 mL
Buffer PRB	15mL	70 mL
RNA Wash Buffer I	30 mL	150 mL
RNA Wash Buffer II (Concentrate)	12 mL	50 mL
DEPC-Treated ddH_2O	10 mL	30 mL
User Menu	1	1

* Add 48 mL (R03-01) or 200 mL (R03-02) 100% ethanol into RNA Wash Buffer II before use.
* **Buffer PLY contains chaotropic salt, not compatible with disinfecting agents contain bleach. Ware gloves and protective eyewear when handling!**

Before use

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

- Determine the volume of Buffer PLY to be used and add 10 µL of ß-mercaptoethanol (ß-ME) per 1 mL Buffer PLY before use. Buffer PLY contains ß-ME can be stored 4 -22°C for up to 1 month.
- Buffer PLY may form precipitates below room temperature. Warm up at 37°C to dissolve before use.
- Add 48 mL (R03-01) or 200 mL (R03-02) 100% ethanol to RNA Wash Buffer II before use.

Materials supplied by users

- Tabletop microcentrifuge and 1.5 mL RNase free tubes.
- Tissue grinder
- Liquid nitrogen
- 100% ethanol
- 70% ethanol

Sample preparation

- Grind plant tissues into a fine powder in liquid nitrogen
- Sample should not be allowed to thaw before adding Buffer PLY.

Extracting Total RNA From Plant Tissue

1. **Weigh 100 mg powdered plant tissue and place into a 2ml microtube. Add 450 µl Buffer PLY, vortex vigorously to make sure that all clumps are dispersed. Incubate at 56°C for 3 min.**
2. **Centrifuge the microtube at 16,000 x g for 3 min. Transfer the supernatant to homogenization column, centrifuge at 16,000 x g for 1 min**
3. **Add 250µl Buffer PRB, mix well by pipetting. Transfer the mixture into a RNA column and centrifuge at 16,000 x g for 1 min. Discard the flow-through and put the column back to the collection tube.**
4. **Add 500 µl RNA Wash Buffer I to the column and centrifuge at 16,000 x g for 1 min. Discard the flow-through.**
5. **Add 500 µl RNA Wash Buffer II to the column and centrifuge at 16,000 x g for 30s. Discard the flow-through and put the column back to the collection tube.**
6. **Centrifuge at 16,000 x g for 1-2 min. Discard the flow-through. The residual ethanol will be removed in this step.**
7. **Place the column to a RNase-free 1.5 mL tube, add 50 µl DEPC-treated water or RNase-free water to the column and centrifuge at 16,000 x g for 1 min. The RNA is in the flow-through liquid. Store the RNA solution at -20°C.**
8. **Optional: Add the eluted RNA solution back to the column and centrifuge at 16,000 x g for 1 min. The first elution normally yields 60-70% of the RNA while the second elution yield 20-30% of the RNA bound to the column.**

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.

Extracting Total RNA From Animal Tissue/Cells

1. **Adherent cells: 3 to 5x10^6 cells are washed once with PBS and detached with Trypsin-EDTA, resuspended in PBS and pellet. For the suspension cells, 3 to 5x10^6 cells are pelleted, resuspended in PBS and pellet. Add 350 µl Buffer PLY, pipet or vortex vigorously to make sure that all clumps are dispersed. Continue to Step 3.**
2. **Animal tissue: weigh 10-30mg animal tissue, place in a 1.5 ml or 2 ml microcentrifuge tube, add 500 µl Buffer PLY, homogenize with a electric homogenizer. Continue to Step 3.**
3. **Transfer the lysis mixture to a homogenization column, centrifuge at 16,000 x g for 1 min, keep the elute.**
4. **Add 350 µl Buffer PRB to the elute, mix well by pipetting. Transfer the mixture into a RNA column and centrifuge at 16,000 x g for 1 min. Discard the flow-through and put the column back to the collection tube.**
5. **Add 500 µl RNA Wash Buffer I to the column and centrifuge at 16,000 x g for 1 min. Discard the flow-through.**
6. **Add 500 µl RNA Wash Buffer II to the column and centrifuge at 16,000 x g for 30s. Discard the flow-through and put the column back to the collection tube. Repeat once.**
7. **Centrifuge at 16,000 x g for 1-2 min. Discard the flow-through. The residual ethanol will be removed in this step.**
8. **Place the column to a RNase-free 1.5 ml tube, add 50 µl DEPC -treated water or RNase-free water to the column and centrifuge at 16,000 x g for 1 min. The RNA is in the flow-through liquid. Store the RNA solution at -20°C.**
9. **Optional: Add the eluted RNA solution back to the column and centrifuge at 16,000 x g for 1 min. The first elution normally yields 60-70% of the RNA while the second elution yield 20-30% of the RNA bound to the column.**

RNA Cleaning

In cases the RNA is extracted with other methods or RNA sample is treated with DNase I to remove genomic DNA contamination. Further RNA cleaning is required.

1. **RNA sample is prepared in RNase-free water at around 1µg/µl. For 50µl RNA sample, add 300 µl Buffer PLY and mix.**
2. **Add 350 µl Buffer PRB to the mixture, mix well by pipetting. Transfer the mixture into a RNA column and centrifuge at 16,000 x g for 1 min. Discard the flow-through and put the column back to the collection tube.**
3. **Add 500 µl RNA Wash Buffer II to the column and centrifuge at 16,000 x g for 30s. Discard the flow-through and put the column back to the collection tube. Repeat once.**
4. **Centrifuge at 16,000 x g for 1-2 min. Discard the flow-through. The residual ethanol will be removed in this step.**
5. **Place the column to a RNase-free 1.5 ml tube, add 50 µl DEPC-treated water or RNase-free water to the column and centrifuge at 16,000 x g for 1 min. The RNA is in the flow-through liquid. Store the RNA solution at -20°C.**
6. **Optional: Add the eluted RNA solution back to the column and centrifuge at 16,000 x g for 1 min. The first elution normally yields 60-70% of the RNA while the second elution yield 20-30% of the RNA bound to the column.**

Troubleshooting

Problem	Possible rea-	Suggested Improvement
Low A260/A280 ratios	Protein contamination	Do a Phenol: Chloroform extraction. Loss of total RNA (up to 40%) should be expected.
	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 11,000 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.
	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.
	Ethanol not added to buffer	Add ethanol to the RNA Wash Buffer and DNase Stop Solution 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.
	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 1-2x105 or increase buffer volume and do multiple loadings to column.

More EasyPrep™ Total RNA Purification Kits

Catalog #	Product Name	Preps
R01-01	Tissue RNA Miniprep kit	50
R01-02	Tissue RNA Miniprep kit	250
R02-01	Blood RNA Miniprep kit	50
R02-02	Blood RNA Miniprep kit	250
R03-01	Plant RNA Miniprep kit	50
R03-02	Plant RNA Miniprep kit	250
R9603-01	96-well Plant RNA Miniprep kit	4x96
R9603-02	96-well Plant RNA Miniprep kit	20x96
R1001-01	RNA Secure Solution	50 mL
R1001-02	RNA Secure Solution	100 mL

Limited use and warranty

This product is warranted to perform as described in its labeling and in Bioland's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Bioland. Bioland's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of Bioland, to replace the products, Bioland 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.

Bioland Scientific LLC

14925 Paramount Blvd., Suite C

Paramount, CA 90723

USA

Tel: (877) 603-8882

Fax: (562) 733-6008

Email: service@bioland-sci.com

order@bioland-sci.com

Visit our web at **www.bioland-sci.com** and learn more about Bioland products