Total RNA Maxi Kit (Plant)

For research use only

Sample size : 500 mg of fresh plant tissue
Yield : 50-300 µg of RNA from young leaf samples
Elution Volume : 500 µl
Format : spin column
Operation time : within 60 minutes

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Introduction

The Total RNA Maxi Kit (Plant) provides an efficient method for purifying total RNA from plant tissue and cells. Samples are ground in liquid nitrogen and then filtered to remove cell debris. In the presence of a binding buffer and chaotropic salt, the total RNA in the lysate binds to the glass fiber matrix of the spin column (1). Optional DNase treatments can be followed to remove unwanted DNA residue. Once any contaminants have been removed using the Wash Buffer (containing ethanol), the purified total RNA is eluted by RNase-free water. The procedure does not require phenol extraction or alcohol precipitation and can be completed within 1 hour. The purified total RNA is ready for use in RT, RT-PCR, Real-time PCR and Northern Blotting.

Quality Control

The quality of the Total RNA Maxi Kit (Plant) is tested on a lot-to-lot basis by isolating total RNA from a 500 mg young leaf sample. The Purified RNA is quantified with a spectrophotometer and checked by electrophoresis.

Kit Contents

Name	IB47350	IB47351
RB Buffer	60 ml	12 ml
PRB Buffer	60 ml	12 ml
W1 Buffer	50 ml	10 ml
Wash Buffer[1] (Add Ethanol)	25 ml (100 ml)	5 ml (20 ml)
RNase-free water	6 ml	1 ml
Filter Column	10 pcs	2 pcs
RB Maxi Column	10 pcs	2 pcs

Order Information

Product Name	Package size	Cat. No.
Total RNA Mini Kit (Tissue)	50/100 preps	IB47301/02
Total RNA Maxi Kit (Tissue)	10 preps	IB47310
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	IB47321/22/23
Total RNA Maxi Kit (Blood/Cultured Cell)	10 preps	IB47330
Total RNA Mini Kit (Plant)	50/100 preps	IB47341/342
Total RNA Maxi Kit (Plant)	10 preps	IB47350
96-Well Total RNA Kit	4/10 x 96 Wells	IB47360/361
miRNA Isolation Kit	100 preps	IB47371
IBI Isolate	100/500 ml	IB47601/602
Vacuum Manifold (Accessories)	1 SET	IB47500

[1]Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume)

Caution

The components contain irritant agents. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615

Total RNA Maxi Kit Plant Protocol

Due to various plant species containing different metabolites, such as polysaccharides, polyphenols and proteins, we provide two lysis buffers. The standard protocol uses RB Buffer for lysis of plant samples. For most common plant species, the buffer system ensures purified RNA with high yields and high quality. Alternatively, PRB Buffer is provided with the kit. The detergent in this lysis buffer is suitable for some plant samples with high polysaccharide content.

- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume)
- Additional requirements: microcentrifuge tubes (RNase free), centrifuge tubes, ß-mercaptoethanol

DNA Residue Degradation options:

► Optional Step 1 (DNA Residue Degradation): Add 100 µl of DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM $MnCl_2$, 50 µg/ml BSA at 25ºC} to the center of the RB Column matrix. Let stand for 10 minutes at room temperature and then proceed to Step 4 Wash.

►Optional Step 2 (DNA Residue Degradation): Add 2 µl of DNase I (2 KU/ml) mixed in a reaction buffer {50 mM Tris-HCl (pH 7.5), 10 mM $MnCl_2$, 50 µg/ml BSA at 25ºC} to the final elution sample. Let stand for 10 minutes at room temperature.

Step 1 Tissue Dissociation	● Cut off 500 mg (up to 1 g) of fresh or frozen plant tissue. ● Grind the sample (in liquid nitrogen) to a fine powder. Transfer the powder to a 15 ml centrifuge tube (some plant samples can be ground without liquid nitrogen).
Step 2 Lysis	● Add **5 ml of RB Buffer (or PRB Buffer)** and 50 µl of ß-mercaptoethanol to the ground sample and mix by vortex. ● Incubate at room temperature for 5 minutes. ● Place a **Filter Column** in a 50 ml centrifuge tube and transfer the sample lysate to the column. ● Centrifuge for 5 minutes at 1,000 x g. ● Discard the **Filter Column** and Proceed to Step 3 RNA Binding.
Step 3 RNA Binding	● Add a ½ volume of absolute ethanol to the clarified filtrate from Step 2 and vortex immediately (eg. add 2.5 ml of absolute ethanol to 5 ml of filtrate). ● Place a **RB Maxi Column** in a 50 ml centrifuge tube. ● Transfer the ethanol-added mixture to the **RB Maxi Column**. ● Centrifuge at 4,000 x g for 5 minutes and discard the flow-through **(if the mixture could not flow past the RB Maxi Column membrane following centrifugation, increase the centrifuge time until the mixture passes completely)**. Optional Step 1: DNA Residue Degradation (see options above)
Step 4 Wash	● Add **4 ml of W1 Buffer** to the center of the **RB Maxi Column**. ● Centrifuge at 4,000 x g for 3 minutes. ● Discard the flow-through and place the **RB Maxi Column** back in the 50 ml centrifuge tube. ● Add **6 ml of Wash Buffer** (ethanol added) to the center of the **RB Maxi Column**. ● Centrifuge at 4,000 x g for 3 minutes. ● Discard the flow-through and place the **RB Maxi Column** back in the 50 ml centrifuge tube. ● Add **6 ml of Wash Buffer** (ethanol added) to the center of the **RB Maxi Column**. ● Centrifuge at 4,000 x g for 3 minutes. ● Discard the flow-through and place the **RB Maxi Column** back in the 50 ml centrifuge tube. ● Centrifuge at 4,000 x g for 10 minutes to dry the column matrix.
Step 5 RNA Elution	● Place the dried **RB Maxi Column** in a clean 50 ml centrifuge tube (RNase free). ● Add **500 µl of RNase-free water** to the center of the column matrix. ● Let stand for 5 minutes or until the water is absorbed by the matrix. ● Centrifuge at 4,000 x g for 5 minutes to elute the purified RNA. Optional Step 2: DNA Residue Degradation (see options above)

Troubleshooting

Problem	Possible Reasons/Solution
Clogged RB Maxi Column	● Insufficient disruption and/or homogenization ● Too much starting material ● Centrifugation temperature too low (should be 20-25ºC)
Low RNA Yield	● Insufficient disruption and/or homogenization ● Too much starting material ● RNA still bound to the RB Maxi Column membrane ● Ethanol carryover
RNA Degradation	● Harvested sample not immediately stabilized ● Inappropriate handling of starting material ● RNase contamination