Total RNA Mini Kit (Plant)

For research use only

Sample size : up to 100 mg of fresh plant tissue or up to 25 mg of dry plant tissue
Yield : 5-30 μg of RNA from young leaf samples
Format : spin column
Operation time : within 30 minutes

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Introduction

The Total RNA Mini 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, 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 Mini Kit (Plant) is tested on a lot-to-lot basis by isolating total RNA from a 25 mg young leaf sample. The Purified RNA is quantified with a spectrophotometer and checked by electrophoresis.

Kit Contents

Name	IB47340	IB47341	IB47342
RB Buffer	3 ml	30 ml	60 ml
PRB Buffer	3 ml	30 ml	60 ml
W1 Buffer	2 ml	30 ml	50 ml
Wash Buffer[1] (Add Ethanol)	1 ml (4 ml)	12.5 ml (50 ml)	25 ml (100 ml)
RNase-free water	1 ml	6 ml	15 ml
Filter Column	4 pcs	50 pcs	100 pcs
RB Column	4 pcs	50 pcs	100 pcs
2 ml Collection Tube	8 pcs	100 pcs	200 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 (see the bottle label for volume) to the Wash Buffer prior to initial use

Caution

RB Buffer contains chaotropic salt which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles and (anti-fog) procedure mask.

Steps to prevent RNase contamination

- During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.
- Disposable plasticware and automatic pipettes should be sterile (RNase-free) and used only for RNA procedures.
- Non-disposable glassware or plasticware should also be sterile (RNase-free).

References

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

Total RNA Mini 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 a variety of plant samples with high polysaccharide content.

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

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	Procedure
Step 1 Tissue Dissociation	• Cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue. • Grind the sample (in liquid nitrogen) to a fine powder. Transfer the powder to a 1.5 ml microcentrifuge tube (RNase-free) (some plant samples can be ground without liquid nitrogen).
Step 2 Lysis	• Add **500 µl of RB Buffer or PRB Buffer** and 5 µl of ß-mercaptoethanol to the ground sample and mix by vortex. • Incubate at 60ºC for 5 minutes. • Place a **Filter Column** in a **2 ml Collection Tube** and transfer the sample mixture to the column. • Centrifuge for 1 minute 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 shake vigorously (e.g. add 250 µl of absolute ethanol to 500 µl of filtrate). • Place a **RB Column** in a **2 ml Collection Tube**. • Transfer the ethanol-added mixture to the **RB Column**. • Centrifuge at 14-16,000 x g for 1 minute (if the mixture could not flow past the RB Column membrane following centrifugation, increase the centrifuge time until it passes completely). • Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**.
	Optional Step 1: DNA Residue Degradation (see options above)
Step 4 Wash	• Add **400 µl of W1 Buffer** into the center of the **RB Column**. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**. • Add **600 µl of Wash Buffer** (ethanol added) to the center of the **RB Column**. • Centrifuge at 14-16,000 x g for 30 seconds. • Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**. • Add **600 µl of Wash Buffer** (ethanol added) to the center of the **RB Column**. • Centrifuge at 14-16,000 x g for 1 minute. • Discard the flow-through and place the **RB Column** back in the **2 ml Collection Tube**. • Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.
Step 5 RNA Elution	• Place the dried **RB Column** in a clean 1.5 ml microcentrifuge tube (RNase-free). • Add **50 µl of RNase-Free Water** to the center of the column matrix. • Let stand for at least 2 minutes to ensure the **RNase-Free Water** is absorbed by the matrix. • Centrifuge at 14-16,000 x g for 1 minute to elute the purified RNA. If higher RNA concentration is required, repeat Step 5 using the final eluate.
	Optional Step 2: DNA Residue Degradation (see options above)

Troubleshooting

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