HiYield™ Total RNA Maxi Kit (Plant)

Cat. No.:	YRPM10	YRPM25
Product Name:	HiYield™ Total RNA Maxi Kit (Plant)	
Reactions:	10	25
Sample:	Up to 500 mg of Fresh Plant Tissue, 125 mg of Dry Plant Tissue	
Yield:	Up to 300 ug	
Format:	Spin Column	
Operation:	Centrifuge	
Operation Time:	Within 60 Minutes	

Introduction

HiYield™ Total RNA Maxi Kit (Plant) provides an efficient method to purify total RNA from plant tissue and cells. Samples are distrusted by grinding in liquid nitrogen and then filtered to remove cell debris. The entire 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.

Features

Ready-to-use RNA for many downstream applications.
Excellent recovery of RNA.
Complete removal of contaminants and inhibitors.

Components

ITEM	YRPM10	YRPM25
RB Buffer	60ml	130ml
PRB Buffer	60ml	130ml
W1 Buffer	50ml	130ml
Wash Buffer*	25ml	62.5ml
RNase-Free Water	6ml	30ml
Lysate Filter Column	10pcs	25pcs
RPM Column	10pcs	25pcs

*Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

Applications

Purified RNA is ready for direct use in RT-PCR, Real-Time RT-PCR, Northern Blotting, Primer Extension, RNase Protection Assays, mRNA Selection, cDNA Synthesis.

Quality Control

The quality of HiYield™ Total RNA Maxi Kit (Plant) is tested on a lot-to-lot basis. The kits are tested by isolation of total RNA from 125 mg of young leaf. The purified RNA could be quantified with a spectrophotometer and checked by agarose gel.

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.

Protocol

Important Technical Information:

Since different plant species contain different metabolites, such as polysaccharides, polyphenolics and proteins, two different lysis buffers (RB Buffer & PRB Buffer) are provided for various samples.

RB Buffer: For most common plant species, RB Buffer system ensures purified RNA with high yields and little degradation.

PRB Buffer: An alternative buffer, PRB Buffer, is also provided with this kit. This lysis buffer is suitable for plant samples containing large amounts of polysaccharides.

In the majority of extractions, both buffer systems should provide adequate results. The researcher may try one buffer system first or both in parallel.

Things to do before starting:

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

Additional requirements: absolute ethanol,centrifuge tubes (RNase-free),ß-mercaptoethanol.

Optional Steps of DNA Residue Degradation:

Optional Step 1:

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 RPM Column matrix. Let stand for 10 minutes at room temperature and then proceed to Step 4 Wash.

Optional Step 2:

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 **Lysate 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 **Lysate Filter Column** and Proceed to Step 3 RNA Binding.
Step 3 RNA Binding	★Add a half 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 **RPM Column** in a 50 ml centrifuge tube. ★Transfer the ethanol-added mixture to the **RPM Column**. ★Centrifuge at 4,000 x g for 5 minutes and discard the flow-through **(if the mixture could not flow past the RPM Column membrane following centrifugation, increase the centrifuge time until the mixture passes completely)**.
	Optional Step 1 of DNA Residue Degradation **(see optional steps of DNA Residue Degradation in page 3)**
Step 4 Wash	★Add **4 ml of W1 Buffer** to the center of the **RPM Column**. ★Centrifuge at 4,000 x g for 3 minutes. ★Discard the flow-through and place the **RPM Column** back in the 50 ml centrifuge tube. ★Add **6 ml of Wash Buffer** (ethanol added) to the center of the **RPM Column**. ★Centrifuge at 4,000 x g for 3 minutes. ★Discard the flow-through and place the **RPM Column** back in the 50 ml centrifuge tube. ★Add **6 ml of Wash Buffer** (ethanol added) to the center of the **RPM Column**. ★Centrifuge at 4,000 x g for 3 minutes. ★Discard the flow-through and place the **RPM 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 **RPM Column** in a clean 50 ml centrifuge tube (RNase-free). ★Add **500 µl of RNase-free water** into the center of the column matrix. ★Let stand for 5 minutes or until the water has been absorbed by the matrix. ★Centrifuge at full speed for 5 minutes to elute the purified RNA.
	Optional Step 2 of DNA Residue Degradation **(see optional steps of DNA Residue Degradation in page 3)**

Troubleshooting

Problem	Possible Reasons/Solution
Clogged RPM 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 RPM Column membrane ★Ethanol carryover
RNA Degradation	★Harvested sample not immediately stabilized ★Inappropriate handling of starting material ★RNase contamination