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HiYield™ Total RNA Mini Kit (Plant)

Cat. No.:	YRP50	YRP100	YRP300
Product Name:	HiYield™ Total RNA Mini Kit (Plant)		
Reactions:	50	100	300
Sample:	Up to 100 mg of fresh plant tissue, up to 25 mg of dry plant tissue		
Yield:	Average RNA yield of 25-30 µg from 50 mg of Thale Cress leaf		
Elution Volume:	50 µl		
Format:	Spin Column		
Operation:	Centrifuge		
Operation Time:	Within 15 Minutes		

Description

HiYield™ Total RNA Mini Kit (Plant) provides an efficient method to purify total RNA from a variety of 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 15 minutes, with an average RNA yield of 25-30 µg from 50 mg of Thale Cress leaf typically. The purified total RNA is ready for use in RT-PCR, real-time PCR, Northern Blotting...etc. For sample amount more than 100 mg of fresh plant tissue or 25 mg of dry plant tissue per preparation, please refer to HiYield™ Total RNA Maxi Kit (Plant).

Features

1. Rapid extraction of total RNA from plant tissue within 15 minutes.
2. Consistent ready-to-use RNA for many downstream applications.
3. Complete removal of contaminants and inhibitors. No phenol, chloroform or alcohol.

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 Mini Kit (Plant) is tested on a lot-to-lot basis. The kits are tested by isolation of total RNA from 50 mg of fresh Thale Cress (Arabidopsis thaliana) leaves. The purified RNA is quantified with a spectrophotometer and the yield of total RNA is more than 25 µg with A260/A280 ratio 1.8 - 2.0. Finally the purified RNA is analyzed by electrophoresis.

Storage

HiYield™ Total RNA Mini Kit (Plant) shall be shipped and stored dry at room temperature (15-25℃).

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Contents

Item	YRP50	YRP100	YRP300
RB Buffer	30 ml	60 ml	160 ml
PRB Buffer	30 ml	60 ml	160 ml
W1 Buffer	30 ml	50 ml	130 ml
Wash Buffer (concentrated)*	12.5 ml	25 ml	100 ml
RNase-free Water	6 ml	15 ml	30 ml
Lysate Filter Column	50 pcs	100 pcs	300 pcs
RP Column	50 pcs	100 pcs	300 pcs
2 ml Collection Tube	100 pcs	200 pcs	600 pcs

* Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.

Important Notes

Please read the entire notes prior to starting any of the protocol procedures.

Caution:

RB Buffer and PRB Buffer contain chaotropic salt. During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.

Reagents to Be Supplied by User:

Microcentrifuge tubes, pipette tips, ß-mercaptoethanol, absolute ethanol, liquid nitrogen.

Things to Do before Starting:

Various plant species contain different metabolites such as polysaccharides, polyphenols, and proteins. The standard protocol uses RB Buffer for lysis of most common plant species. The RB Buffer system ensures purified RNA with high yields and high quality. Alternatively, PRB Buffer is provided with the kit to ensure efficient cell lysis of plant species with high polysaccharide content.

Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.

Steps to Prevent RNase Contamination:

Non-disposable glassware, plasticware, automatic pipettes and disposable plasticware should be sterile (RNase-free) and used only for RNA procedures.

DNA Removal Options:

For DNA-free RNA, perform optional steps listed in the protocol. Either follow the steps after RNA Binding, or steps after RNA Elution.

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Protocol

Please read the entire important notes prior to starting.

Step 1 Sample Preparation	1. Cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue. 2. Freeze the sample with liquid nitrogen 3. Grind the sample to a fine powder then transfer it to a 1.5 ml microcentrifuge tube. NOTE: Some plant samples can be ground sufficiently in the absence of liquid nitrogen.
Step 2 Cell Lysis	1. Add 500 µl of RB Buffer or PRB Buffer and 5 µl of ß-mercaptoethanol (or 10 µl of freshly prepared 2M Dithiothreitol in RNase Free Water) and mix by vortex. 2. Incubate at 60ºC for 5 minutes. Place a Lysate Filter Column in a 2 ml Collection Tube. 3. Transfer the sample mixture to the Lysate Filter Column. 4. Centrifuge for 1 minute at 1,000 x g then discard the Lysate Filter Column. 5. Carefully transfer the clarified filtrate to a new 1.5 ml microcentrifuge tube.
Step 3 RNA Binding	1. Add a ½ volume of absolute ethanol to the clarified filtrate then shake vigorously. E.g. Add 250 µl of absolute ethanol to 500 µl of filtrate. 2. Place a RP Column in a 2 ml Collection Tube then transfer the mixture to the RP Column. Centrifuge at 14,000-16,000 x g for 1 minute. NOTE: If the mixture could not flow past the RP Column membrane following centrifugation, increase the centrifuge time until it passes completely. 3. Discard the flow-through then place the RP Column back in the 2 ml Collection Tube.
(Optional) DNA Removal Option 1: In Column DNase I Digestion	The amount of DNA contamination is significantly reduced following In Column DNase I Digestion. However, traces of residual DNA may be detected in very sensitive application. In this situation, please perform Optional Step 2: DNA Digestion In Solution instead to efficiently remove trace amounts of DNA. Standard DNase buffers are incompatible with In Column DNase I Digestion and may affect RNA integrity and reduce yield. 1. Add 400 µl of Wash Buffer (make sure ethanol was added) to the RP Column then centrifuge at 14,000-16,000 x g for 30 seconds. 2. Discard the flow-through and place the RP Column back in the 2 ml Collection Tube. 3. Prepare DNase I solution in a 1.5 ml microcentrifuge tube (RNase-free) as follows: DNase I — 5 µl (2 U/µl) DNase I Reaction Buffer — 45 µl Total Volume — 50 µl 4. Gently pipette DNase I solution to mix (DO NOT vortex) then add DNase I solution (50 µl) into the CENTER of the RP column matrix. 5. Incubate the column for 15 minutes at room temperature (20-30ºC) then proceed with Step 4 RNA Wash.

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Step 4 **Wash**	1. Add 400 µl of W1 Buffer into the center of the RP Column. 2. Centrifuge at 14,000-16,000 x g for 30 seconds. 3. Discard the flow-through then place the RP Column back in the 2 ml Collection Tube. 4. Add 600 µl of Wash Buffer (make sure ethanol was added) to the center of the RP Column. Centrifuge at 14,000-16,000 x g for 30 seconds. 5. Discard the flow-through then place the RP Column back in the 2 ml Collection Tube. 6. Add 600 µl of Wash Buffer (make sure ethanol was added) to the center of the RP Column. Centrifuge at 14,000-16,000 x g for 1 minute. 7. Discard the flow-through then place the RP Column back in the 2 ml Collection Tube. 8. Centrifuge at 14,000-16,000 x g for 3 minutes to dry the column matrix.
Step 5 **RNA Elution**	1. Place the dried RP Column in a clean 1.5 ml microcentrifuge tube (RNase-free). 2. Add 50 µl of RNase-free Water into the CENTER of the column matrix. Let stand for at least 2 minutes to ensure the RNase-free Water is completely absorbed by the matrix. 3. Centrifuge at 14,000-16,000 x g for 1 minute to elute the purified RNA. NOTE: If higher RNA concentration is required, repeat Step 4 using the final eluate.
(Optional) **DNA Removal Option 2: DNA Digestion In Solution**	1. Prepare DNase I reaction in a 1.5 ml microcentrifuge tube (RNase-free) as follows: RNA in RNase-free Water 1-40 µl DNase I 0.5 µl/µg RNA DNase I Reaction Buffer 5 µl RNase-free Water Add to final volume = 50 µl Total Volume 50 µl 2. Gently pipette the DNase I reaction solution (DO NOT vortex) then incubate the microcentrifuge tube at 37ºC for 15-30 minutes. 3. Stop the reaction by adding 1 µl of 20 mM EGTA (pH=8.0) then incubate the microcentrifuge tube at 65℃ for 10 minutes. NOTE: DNase I Reaction Buffer may cause aberrant migration or smearing of RNA on gels. If analyzing RNA by gel electrophoresis, repurify the RNA sample by using the HiYield™ RNA UltraPurification Kit (Cat. No. YUR100 / YUR300) instead of stopping the reaction with EGTA

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Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	1. Insufficient disruption and/or homogenization. 2. Too much starting material. 3. Centrifugation temperature was too low (must be between 20ºC to 25ºC).
Low RNA Yield	1. Insufficient disruption and/or homogenization. 2. Too much starting material. 3. RNA still bound to RP Column membrane. 4. Ethanol carryover.
RNA Degradation	1. Harvested sample not immediately stabilized. The harvested sample should be stabilized immediately prior to use. 2. Inappropriate handling of starting material. 3. RNase contamination: 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).

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