RNAs-ici!-R

RS-0003N

～RNA extraction buffer～

For Rosaceae plant leaves with viscous substance

User Manual

Ver. 1.0

RIZO Inc.

Table of Contents

	Page
Key Features	2
Kit Components	2
Expiry and Storage Conditions	2
Safety Warnings and Precautions	2
Reagents and Equipment Required	3
Preparation before RNA Extraction	4
Protocol for RNA Extraction	5-7
Troubleshooting	8
Examples	9
Contacts	Back Cover

Key Features

RNAs-ici!-R is a specialized RNA extraction buffer ideal for plant tissues containing viscous substance, such as pectin or mucosaccharide.
Extracted RNA contains genomic DNA. Please perform DNase treatment and/ or LiCl precipitation as needed for downstream applications.*
This product is accompanied by *'Acidified phenols-ici!'*, which is convenient to prepare acidified phenol, which is useful for effective RNA extraction.

*DNase I or LiCl is not included in this product.

Kit Conponents

1. *RNAs-ici!-R* 90 ml（for 150 extractions）
   color：pale orange
2. *RNA precipitation solution*（50 ml）*
3. *Acidified phenols-ici!* 40 ml（for 80ml acidified phenol）
   color：pink

*Add 50ml 2-propanol before use.

Expiry and Storage Conditions

Store at 4℃.
Expiry: 6 months after opening.

Safety Warnings and Precautions

For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals. Handling by persons other than those who have basic knowledge of RNA operation and reagents is prohibited.

*Contents of the leaflet, specification and price of the product are subject to change without notice.

Reagents and Equipment Required

Reagents

Phenol, crystal*
Chloroform
2-mercaptoethanol or 2M dithiothreitol (DTT)
2-propanol
70% ethanol**
DEPC water

* For preparing acidified phenol.(acidified phenol can be substituted by water-saturated phenol in your lab, but we recommend to prepare it using acidified phenols-ici!)

** Ethanol(molecular biology grade) : nuclease free water=7:3(vol:vol)

Equipment

High-speed refregerated micro centrifuge

Others

1.5 ml tube
Micro pipettes (1,000 μl, 200 μl)
Pipette tips
Mortar or microtube pestle

Preparation before RNA Extraction

Preparation of Acidified Phenol (please ware safety globes)

1. Transfer '*Acidified phenols-ici!*' into clean beaker (do not discard), empty the bottle.

2. Put phenol crystal into the bottle to eight-tenth full. Warm the bottle in 65℃ water to liquidify phenol (approx. 60m l liquid phenol).

3. Put '*Acidified phenols-ici!*' of 1. Into the bottel of 2.. Fasten cap and shale well.

4. Leave the bottle for a while until the liquid separates into two layers. Lower layer (pink) is the acidified phenol (approx. 80ml acidified phenol can be prepared).

5. Store at 4℃ after preparation.

Preparation of RNA Precipitation Solution

Add 50ml 2-propanol to *RNA precipitation solution* (50 ml) and mix well. Use this prepared *RNA precipitation solution* in extraction procedure illustrated from next page.

Protocol for RNA Extraction

1. Put 600μl *RNAs-ici!-R* into a clean tube(1.5ml). Add 24μl 2-mercaptoethanol (alternatively, add 12μl 2M DTT).

2. Pulverize samples (10～50 mg[NOTE1)]) with mortar and pestle in liquid nitrogen and transfer into buffer with frozen spatula,. Mix with buffer immediately .

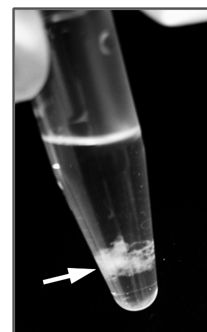
3. Homogenize samples using voltex or microtube pestle.

4. Keep on ice for 5 min.

5. Add 500μl acidified phenol, 200μl chloroform and mix well. Keep on ice for 15min.

6. Centrifuge at 15,000 rpm for10 min. at 4℃.

7. Transfer 400 μl supernatant into fresh clean tube. Add 400μl ethanol. Mix well.*
   * Viscous substance appears as precipitate in case of viscous substance-rich materials (see photo).



8. Centrifuge at 3,000 rpm for 1 min.[NOTE3)] at room temperature (20-25℃) .

9. Carefully avoiding precipitate (viscous substance), transfer 600μl supernatant into fresh clean tube. Add 600μl RNA precipitation solution. Mix well by inversion. Keep on ice for 15 min.

10. Centrifuge at 15,000 rpm for 10min.[NOTE4)]

11. Discard the supernatant.[NOTE5)] Add 400μl 70% ethanol.

12. Centrifuge at 15,000 rpm for 10min.

１３. Discard the supernatant. Dry pelette. NOTE6)

１４. Add 20～50μl DEPC water, mix well. NOTE7) Use RNA for further application.

***For 1)-6), see NOTES on page 7.**

NOTES

1) Too much amount of starting materials may cause low RNA yield and/or quality.

2) Pulverize samples with mortar and pestle in liquid nitrogen and transfer into buffer with frozen spatula, in order to keep samples frozen. It is important to mix with buffer before thawing. Soft materials can be directly added into *RNAs-ici!-R* buffer on ice and crushed by hand homogenizer or micropestle .

3) Do not centrifuge more than 1 min. It may cause low RNA yield. Depending on samples, shorter (e.g. ～15sec.) centrifuge will make effective extraction.

4) Arrange the direction of tubes at centrifuge, in order to check RNA pellet easily (it may be very difficult to see RNA pellet).

5) Be careful not to wash out RNA pellet.

6) Overdrying may make RNA difficult to dissolve into water.

7) Adjust the amount of DEPC water according to properties of samples and downstream research application.

Technical Tips

Extracted RNA contains genomic DNA. Please perform DNase treatment and/ or LiCl precipitation as needed for downstream applications.

This protocol is devised for RNA extraction from ～50mg sample. Scaling up is possible for large amount of samples.

Troubleshooting

Troubles	Possible causes	Suggestions
Low RNA yield	Insufficient pulverizing and homogenization of materials.	Pulverize materials as fine as possible before adding into the extraction buffer.
	RNA degrades in materials before extraction.	Pulverize materials in liquid nitrogen to keep materials frozen, and mix with extraction buffer before melting.
	To much starting materials causes insufficient RNase inhibition by extraction buffer.	Reduce amount of starting materials to ~50mg.
Too much DNA contamination	Extracted RNA contains genomic DNA. Please perform DNase treatment and/ or LiCl precipitation as needed for downstream applications.	

Examples

RNA extraction from plant tissues containing viscous substance.

RNA was extracted from 50mg of plant tissues listed below using *RNAs-ici!-R*.

（samples）

Flower petals

1. Rose
2. Chrysanthemum
3. Florists' chrysanthemum

Leaves

4. Rose
5. Strawberry

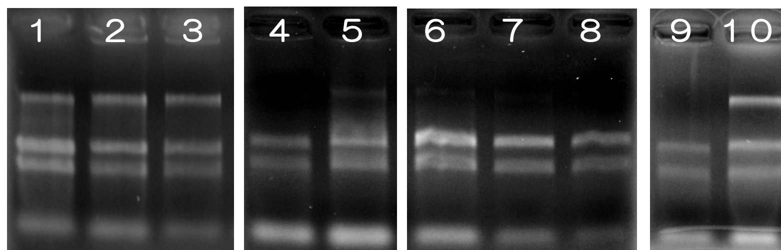
Fruits

6. Strawberry
7. Pear
8. Apple

etc.

9. Taro
10. Green onion

0.8% Agarose /0.5×TBE



Extracted RNA contains genomic DNA. Please perform DNase treatment and/ or LiCl precipitation as needed for downstream applications.

Contacts

RIZO Inc.

Amakubo 2-9-2 Tsukuba, Ibaraki, JAPAN

Tel ; +81-29-852-9351

E-mail ; info@rizo.co.jp

URL ; http://rizo.co.jp/

Copyright ©2012 RIZO Inc. All Right Reserved.