RNAs-ici!-S

RS-0001N

~RNA extraction buffer~

For starch or polysaccharide -rich plant tissue

User Manual

Ver. 1.4

RIZO Inc.

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Key Features

RNAs-ici-S is a specialized RNA extraction buffer ideal for plant tissues containing starch or polysaccharide, such as cereals, beans, tuber, fruits, *etc.*
RNAs-ici!-S provides speedy and low-cost RNA extraction in a few handling steps without using extraction columns.
Extracted RNA can be used for real time-PCR, Northern blot analysis, and poly-A RNA extraction.
This product is accompanied by 'Acidified phenols-ici!' , which is convenient to prepare acidified phenol, which is useful for effective RNA extraction.
Extracted RNA contains genomic DNA. Please perform DNase treatment and/ or LiCl precipitation as needed for downstream applications.*

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

Kit Conponents

RNAs-ici!-S 85 mL（for 210 extractions）
color：yellow
Acidified phenols-ici! 40ml（for 80ml acidified phenol）
color：pink

Storage conditions

Store at 4℃ after opening.

Safety Warnings and precautions

The use of this product is for research use only, not recommended or intended for diagnosis of disease in humans or animals. Do not use internally in humans or animals.

*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㎕, 200㎕)
Pipette tips
Mortar or microtube pestle

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.

Protocol for RNA Extraction

1. Put 400μl *RNAs-ici-S* into a clean tube (1.5ml). Keep on ice. Add 4μl 2-mercaptoethanol (alternatively, add 4μl 2M DTT).

2. Pulverize samples using mortar and pestle or electric mixer .[Note1)] Add 50～100 mg[Note2)] pulverized samples into buffer in tube. Mix immediately.

3. Homogenize samples using voltex or microtube pestle.

4. Add 200μl acidified phenol. Mix well.

5. Add 150μl chloroform and mix well. Keep on ice for 15 min.

6. Centrifude at 15,000 rpm for10 min.

7. Prepare 1.5 m l tube containing 300 μl 2-propanol on ice.Transfer ～300 μl of supernatant. Mix well. Incubate -20℃ for 30 min.

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

9. Discard the supernatant,.[Note3)] Add 400μl 70% ethanol.

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

11. Discard the supernatant,. Dry pelette. [Note4)]

12. Add 20～50 μl DEPC water, mix well. [Note5)] Use RNA for further application.

***For 1)-5), see NOTES on page 6**

NOTES

1) Hard materials such as grains can be wrapped in aluminiun foil and pulverized with pliers. Soft materials can be directly added into RNAs-ici!-S buffer and crushed by hand homogenizer or micropestle. For frozen samples, pulverize in liquid nitrogen and transfer into buffer with frozen spatula, in order to keep samples frozen. It is important mix with buffer at the moment of melting.

2) Too much amount of starting materials may cause low RNA yield and/or quality. For example, 5 rice grains weighs approx.100mg.

3) Be careful not to wash out RNA pellet.

4) Overdrying may make it difficult for RNA to dissolve into water.

5) Amount of DEPC water varies according to properties of samples and downstream research application.

Technical Tips

Additional treatment of step 4-step 6 may improve RNA quality when extracted RNA contains too much contaminants.

This protocol is devised for RNA extraction from 50-100mg sample. Scaling up is possible for larger amount of samples.

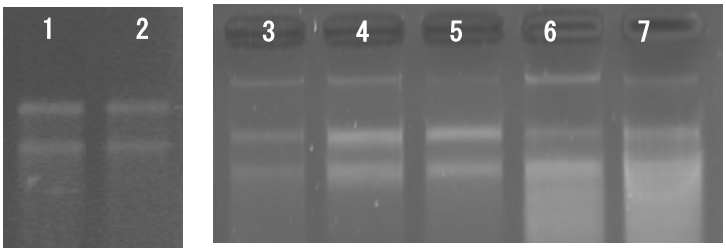
Troubleshooting

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. In case it's difficult to pulverize well, homogenize materials in the buffer throughly using micropestles or pipette tips.
	RNA degrades in materials before extraction.	Pulverize materials in liquid nitrogen to keep materials frozen, and mix with extraction buffer before melting.
Too much DNA contamination	To much starting materials	Reduce amount of starting materials to approx. 100mg.
	Inefficient acidified phenol extraction.	DNA contamination can be reduced by repeating acidified phenol treatment. （Perform DNase treatment to completelyremove DNA）

Examples

RNA extraction from plant tissues containing starch.

RNA was extracted from 100mg of plant tissues listed below using *RNAs-ici!-S*.



1 Brown rice　2 Polished rice　3 Barley(grain)　4 Ginger（root）
5 Black rice　6 Potate（tuber）　7 Sweet potate（root）

Extracted RNA contains genomic DNA. Please perform DNase treatment as needed for downstream applications.

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