RNAs-ici!-P

RS-0002N

~RNA extraction buffer~

For polyphenol-rich plant tissues

User Manual

Ver. 1.0

RIZO Inc.

Table of Contents

Key Features

RNAs-ici!-P is a specialized RNA extraction buffer ideal for polyphenol-rich plant tissues.
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

RNAs-ici!-P 85 mL（for 170 extractions）
color：colorless
Acidified phenols-ici! 40 ml（for 80ml acidified phenol）
color：pink

Expiry and Storage Conditions

Store at room temperature in dark place. Avoid heat and humidity.
When crystals observed warm the buffer to 28-30℃ to resolve crystals before use.
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㎕, 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 500μl *RNAs-ici!-P* into a clean tube(1.5ml). Add 20μl 2-mercaptoethanol (alternatively, add 10μl 2M DTT).

2. Pulverize samples (~50 mg[NOTE1)]) with mortar and pestle in liquid nitrogen and transfer into buffer with frozen spatula,. Mix with buffer immediately .[NOTE2)]

3. Homogenize samples using voltex or microtube pestle.

4. Keep on ice for 5 min.

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

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

7. Prepare 1.5m l tube containing 200㎕ 2-propanol on ice.Transfer ~200 ㎕ of supernatant. Mix well. Incubate -20℃ for 30 min.

8. Centrifuge at 15,000 rpm for 10min.[NOTE3)]

9. Discard the supernatant,.[NOTE4)] Add 400㎕ 70% ethanol.

10. Centrifuge at 15,000 rpm for 10min. at 4℃.

11. Discard the supernatant,. Dry pelette. [NOTE5)]

12. Add 50㎕ DEPC water, mix well. [NOTE6)] Use RNA for further application.

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

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 mix with buffer at the moment of thawing. Soft materials can be directly added into RNAs-ici!-P buffer and crushed by hand homogenizer or micropestle.

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

4) Be careful not to wash out RNA pellet.

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

6) 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.	

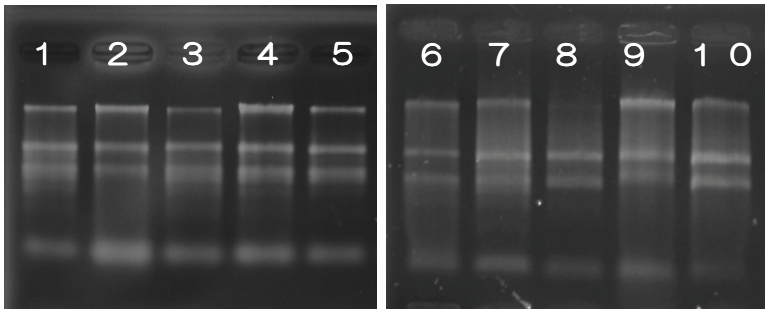
Examples

RNA extraction from polyphenol-rich plant tissues (herb and vegetables)

RNA was extracted from 50mg or 100mg(*) of plant tissue listed below using *RNAs-ici!-P.*

1. Basil
2. Rosemary
3. Lemon balm
4. Asitaba (Angelica keiskei)
5. Purple chicory
6. Rice leaf (purple)
7. Lavender
8. Lotus root*
9. Purple broccoli*
10. Gobo (burdock)

0.8% Agarose /0.5×TBE



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

RNAs-ici!-P

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