RNAs-ici!-F

RS-0005

～RNA extraction buffer～

For body surface mucosas and tissue of fishes

User Manual

Ver. 1.01

RIZO Inc.

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Key Features

RNAs-ici-F is a specialized RNA extraction buffer ideal for mucous membranes and tissues of fishes.
Samples of RNA extraction can be obtained by gently rubbing the body surface of living fish using swabs without wounding fish bodies.
RNAs-ici!-F is also suitable for DNA extraction from fins, muscles or internal organs.
RNAs-ici!-F provides speedy, low-cost and high-yield RNA extraction with a small number of handling steps.
*This product does not contain DNase I. Please perform DNase I treatment and/ or LiCl precipitation as needed for downstream applications.

Kit Conponents

RNAs-ici!-F 21mL（for 50extractions）
color : yellow
Acidified Phenols-ici! 20ml（for 40ml acidified phenol）
color : pink

Storage Conditions

Store at 4℃ after opening.
When crystals observed warm the buffer to 28-30℃ to resolve crystals before use.

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
99.5% ethanol

* 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
Heat block or water bath

Others

1.5 ml tube
Micro pipettes (1,000 μl, 200 μl)
Pipette tips
Swabs (for body surface mucosa)
Stout scissors(for body surface mucosa)

Preparation before RNA Extraction

Preparation of *RNAs-ici!-F*

1. Add 10ml 99.5% ethanol into *RNAs-ici!-F*. Mix well (Check box on label of the bottle).

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. 30ml 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. 40ml acidified phenol can be prepared).

5. Store at 4℃ after preparation.

Protocol for RNA Extraction

1. Put 600μl *RNAs-ici!-F* into1.5mlmicrotube. Add 6μl 2-mercaptoethanol (or 6μl 2M DTT), mix well and keep on ice.

2. Gently rub down the surface of fish with a swab. In case starting materials are gills, internal organs or muscle, remove 5 ～50mg of fresh tissue.

3. Put swab of step 2. into tube of 1., and quickly mix with

RNAs-ici!-F buffer. Cut off the swab and fasten cap. In case material is tissue, put it into tube of 1. and immediately mix with *RNAs-ici!-F* buffer. [NOTE1)]

4. Add 300 μl acidified phenol and mix well. Keep on ice for 10 min.

5. Add 200 μlchroloformand mix well. Keep on ice for 10 min.

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

7. Transfer 300 μl supernatant into fresh clean tube. Add 300 μl isopropanol. Mix well.

8. Centrifuge at 15,000 rpm for10min.

9. Discard supernatant,[NOTE2)] add 500 μl 70% ethanol.

10. Centrifuge at 15,000 rpm for10min.

11. Discard the supernatant,. Dry pelette. [NOTE3)]

12. Add 50 μl DEPC water, mix well.[NOTE4)] use RNA for further application.

***For 1)-4), see NOTES on page 6.**

NOTES

1) Pulverize samples with micropestle if necessary. For frozen samples, pulverize samples with mortar and pestle in liquid nitrogen and transfer into buffer with frozen spatula.

2) Be careful not to wash out RNA pellet.

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

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

Technical Tips

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 sample quantity.	Remove body surface mucosa on overall swab.
	RNA degrades in materials before extraction.	Body surface mucosa should be put into *RNAs-ici!-F* buffer immediately after sampling. RNA may degrade before extraction procedure depending on storage condition.
	Insufficient RNase inhibition by extraction buffer.	Increase 2-mercaptoethanol up to 12μl.
Too much protein contamination	Too much starting materials.	Reduce amount of starting materials to ～10mg.
	Sample contains too much proteins.	To remove proteins, add *RNAs-ici!-F* buffer of step1 to RNA solution to 600μl and follow step 4～.

Example

RNA extraction from body surface mucosa of freshwater fish (goldfish).

RNA was extracted from body surface mucosa of gold fish using *RNAs-ici!-F*.

RNA was extracted from a swabful of body surface mucosa in accordance with the protocol. Then, 15μl each of extracted RNA was electrophoresed.

Analysed with spectrophotometer showed approx. 5.8μgtotal RNA was obtained from a swabful of body surface mucosa.
（RNA extracted by this reagent contains genomic DNA）

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