DNAs-ici!-R

DS-0003N

～DNA extraction buffer～

For *Rosaceae* plant leaves with viscous substance

User Manual

Ver. 1.1

RIZO Inc.

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Key Features

DNA-ici!-R is a specialized DNA extraction buffer ideal for *Rosaceae* plant leaves*. It is best fitted for samples which contain lots of polyphenols, are prone to browning, and show high viscosity when homogenized (e.g. strawberry leaves and rose leaves).

Obtained DNA can be used in a variety of downstream applications.

*DNA in processed foods may not be extracted depending on processing conditions.

Kit Components

DNAs-ici!-R DNA extraction buffer　85 mL
(for 110 extractions)

Additives A（powder）　 2 bottles
Additives B （solution） 10 mL×1 bottle

Expiry and storage conditions

Store the buffer refrigerated at 4℃.

Expiry:

Additives A/B:　 1 month after mixing*.
DNA extraction buffer: 6 months after opening

＊ Mixed additives can be frozen for long-term storage.

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 DNA operation and reagents is prohibited.

*Contents of this leaflet, specification and prices of this product are subject to change without notice.

Reagents and Equipment Required

Reagents

2-propanol
Phenol：chloroform（1：1、v/v）*
70% Ethanol*
TE （10 mM Tris-HCl, 1 mM EDTA, pH 8.0） or
Nucease-free water

*Use a mixture of:
[tris-saturated phenol which is made by saturating crystal phenol with tris buffer(pH8.0)]:[chloroform] =1:1 (vol:vol)
Products with volume ratio of (phenol:chloroform:Isoamyl alcohol=25:24:1) can be a substitution. (e.g. SIGMA's Cat.No. P2069)
*Ethanol（molecular biology grade）：nuclease-free water =7：3(vol:vol)

Equipment

High speed refrigerated microcentrifuge

Others

1.5 ml tube
Micro pipettes（1,000 μl、200 μl）
Pipette tips

Before use

Put 5ml of additives B (blue label) into additives A (red label) and mix well*. Prepared additives solution shall be added to *DNAs-ici!-R* immediately before extracting DNA.

*ATTENTION! Prepared additives solution expires1month after mixing. Please keep it refrigerated at 4℃ in the dark till it is used.

Protocol for DNA Extraction

Make a solution by mixing 720 μl of *DNAs-ici!-R* with 80μl of prepared additives[Note1)] per sample. Make this as many as number of samples.

1. Put 400μl of *DNAs-ici!-R* with additives into a clean tube(1.5ml) [Note2)] and add 10-50mg of samples.[Note3,4)]

2. Homogenize samples using a microtube pestle. [Note5)]

3. Add a further 400 μl of *DNAs-ici!-R* with additives. Mix well.

4. Add 500 μl of phenol:chloroform (1：1,v/v). Mix well.

5. Centrifuge at 15,000 rpm for 10 min, at room temperature. (20-25℃)

6. Transfer 600μl of supernatant to a clean tube (1.5 ml). Add 200 μl (1/3 vol of supernatant) of 2-propanol. Mix well.

7. Centrifuge at 15,000 rpm for 10 min at room temperature （20-25℃）.

8. Transfer 600 μl of supernatant to a clean tube(1.5 ml). Add 300 μl（1/2 vol of supernatant）of 2-propanol. Mix well.

9. Centrifuge at 15,000 rpm for 10 min at room temperature. (20-25℃)

10. Discard supernatant[6)]. Add 1000μl of 70% ethanol.

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

12. Discard supernatant. Dry pellet.[note7)]

13. Dissolve pellet in 50～100μl of TE or nuclease-free water[Note8)]. Serve contained DNA as a template for PCR.

＊For 1)-7.), see NOTES on page 5

NOTES

1) Add prepared additives to *DNAs-ici!-R* immediately before use. Don't use those which elapsed a day or more after mixing.

2) In case of cryopreserved tissues, the sample needs to be dipped into extraction buffer before thawing. Too much samples may cause low DNA yield and/or quality, leading to inhibition of PCR amplification.

3) Avoid fully extended leaves. Use as newly extended young leaves as possible. In case of fully extended leaves, developed cell walls block sufficient homogenization, and accumulated polyphenol compounds may lead to low DNA yield and quality as a consequence.

4) In case of raw leaves, depending on the sample, it may be easier to handle homogenization by adopting about half amount of extraction buffer shown on the protocol. In this case, after homogenization, add extraction buffer(*DNAs-ici!-R* ＋Additives solution) to make the total amount 800 μl and flop upside down.

5) 1,000μl pipette tips with tip holes closed by burning over alcohol lamps/lighters are good enough as homogenizer.

6) Be careful not to wash out DNA pellet.

7) Overdrying may make it difficult for DNA to dissolve into TE or water.

8) Optimal amount of TE or water varies according to the properties of materials (species, organ, tissue, or conditions) and PCR conditions (reaction volume, polymerase or reaction program). Adjust the amount of reagents according to the conditions above.

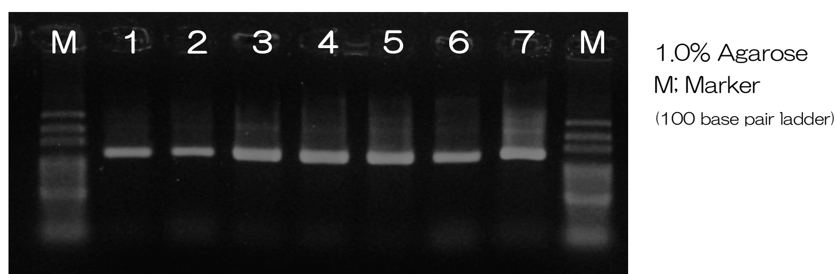
The protocol above is devised for DNA extraction from samples of very small amount.

Troubleshooting

Trouble	Suspected causes	Suggestions
Low DNA yield	Insufficient grinding and homogenization of materials.	Homogenize the sample as thoroughly as possible.
	Insufficient DNA elution from samples into extraction buffer.	Homogenize the sample with *DNAs-ici!-R* and flop upside down well before proceeding to the next step.
Yielding large amount of white precipitates after adding isopropanol, which remains even after washing out with 70% ethanol.	Too much protein and/or lipid content in starting materials.	Do step 4 and 5 of DNA extraction protocol over again and remove proteins and lipids.

Examples

DNA was extracted from *Rosaceae* plant leaves, using *DNAs-ici!-R*. Then, PCR amplification was performed with obtained DNA as templates, using 18S rRNA gene detection primer pair(amplification size is 1,131 bp).



1.0% Agarose
M: Marker
(100 base pair ladder)

1 rose (leaf)
2 strawberry (leaf)
3 apple (leaf)
4 Japanese cherry (leaf)
5 Japanese plum (leaf)
6 soldum (leaf)
7 prune (leaf)

(DNA was extracted from approx. 50mg of raw leaf tissues)

(Composition of reaction)

Template DNA*	1～6 (μl)
10×Buffer	3
dNTP mixture (2.0mM each)	3
primer (4 pmol each/μl)	3
Taq** (5units/μl)	0.25
H_2O	
Total	30 μl

*2-120fold dilution of obtained DNA was used.
**Stratagene Paq5000 DNA polymerase was used.

(cycling program)

95℃ 2 min.
94℃ 35 sec. ⎫
55℃ 30 sec. ⎬ 35 cycles
72℃ 75 sec. ⎭
72℃ 7 min.

Product Line-up

[DNA extraction buffer "*DNAs-ici!*" series]

For starch-rich samples s*DNAs-ici!-S* DS-0001N (420 extractions) *Rice (brown/polished), wheat, chestnuts, Japanese millets, beans, other grains, tubers and roots, cooked rice, flours…etc.*	For plant tissues containing viscous substance *DNAs-ici!-VS* DS-0004 (110 extractions) *Green onion, satoimo (taro), mekabu (thick wakame leaves), natto, aloe…etc.*
For polyphenol-rich samples *DNAs-ici!-P* DS-0002N (230 extractions) *Black rice, red rice, pulses, tea, coffee, herbs, spices, leafy greens, violet vegetables, red wine.…etc.*	For environment-related materials such as soil and activated sludge *DNAs-ici!-E* DS-0008 (100 extractions) *Soil including volcanic ash, leaf molds, activated sludge*
For lipid-rich plant seeds *DNAs-ici!-L* DS-0006 (180 extractions) *Peanuts, almonds, soy beans, walnuts, cashew nuts*	For woods/dried plant tissues *DNAs-ici!-W* DS-0009 (110 extractions) *Wood, bamboo products, straws, rushes, rice hulls…etc.*
For processed foods *DNAs-ici!-PF* DS-0007 (140 extractions) *Tempura Agedama, tortilla chips, Korean miso, Japanese miso, freeze-dried tofus, pasta, soba noodles, udon noodles…etc.*	For body surface mucosa and tissues of fishes *DNAs-ici!-F* DS-0005 (210 extractions) *Various types of mucosa/tissues, saliva, meats, dried seafood…etc.*

[RNA extraction buffer "*RNAs-ici!*" series]

For starch-rich samples *RNAs-ici!-S* RS-0001N (210 extractions) *Endosperm of cereal crops such as rice, wheat, beans (unripe/full-ripe). Vegetative reproduction organs. Roots.*	For Rosacea plant leaves with viscous substance *RNAs-ici!-R* RS-0003N (150 extractions) *Apples, pears, peaches, strawberries and cherries (leaves/petals/fruits). Petals of chrysanthemums, welsh onions and taros.*
For polyphenol-rich samples *RNAs-ici!-P* RS-0002N (170 extractions) *Herbs, Angelica keiskeis, leaves of purple rice, black rice, arctium lappas, lotus roots…etc.*	For body surface mucosa and tissues of fishes *RNAs-ici!-F* RS-0005 (50 extractions) *Body surface mucosal cells, gene expression studies of epizoic microbes…etc.*

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