DNAs-ici!-P

DS-0002N

~DNA extraction buffer~

For polyphenol-rich plant materials

User Manual

Ver. 1.0

RIZO Inc.

Table of Contents

	Page
Key Features	2
Kit Components	2
Storage Conditions	2
Safety Warnings and Precautions	2
Reagents and Equipment Required	3
Before use	3
Protocol for DNA Extraction	4-5
Troubleshooting	6
Examples	7
Contacts	Back Cover

Key Featues

DNAs-ici!-P is a specialized DNA extraction buffer best suited for plant tissues containing polyphenols.

DNAs-ici!-P provides speedy, low-cost and safe DNA extraction, without purification via spin-column nor by phenol/chloroform treatment,.
Obtained DNA can be used in a variety of downstream applications.
*DNA in processed food may not be extracted depending on processing conditions.

Kit Components

DNAs-ici!-P DNA extraction buffer 85 mL
(for 230 extractions)

Additives A（powder） 2 bottles
Additives B（solution） 5 mL×2 bottles

Expiry and storage conditions

Store the buffer refrigerated at 4℃
Expiry:
Additives A/B: 1 month after mixing*.
*Mixed additives can be frozen for long-term storage.

Safety Warning 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 price 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 component ratio of (phenol:chloroform:Isoamyl alcohl=25:24:1) can be a substitution. (i.e SIGMA’s Cat.No. P2069)

*Ethanol（molecular biology grade）：nuclease-free water

=7：3(vol:vol)

Equipment

Microcentrifuge (with rotor for 2ml tubes)

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 expires1month after mixing. Please keep it refrigerated at 4℃ in the dark till it is used (Mixed additives can be frozen for long term storage).

Protocol For DNA Extraction

1. Put 360μl of *DNAs-ici!-P* and 40μl of prepared additives. into a clean tube(1.5ml) NOTE1,2) and add 10-50mg of samples.NOTE3)
2. Homogenize samples using a microtube pestle. NOTE4)
3. Add 400 μl of phenol:chloroform (1 : 1,v/v). Mix well.
4. Centrifuge at 15,000 rpm for 10 min, at room temperature.(20-25℃)
5. Transfer 200 μl of supernatant to a clean tube(1.5 ml) and add 200 μl (equal volume) of 2-propanol. Mix well.
6. Centrifuge at 15,000 rpm for 10 min, at room temperature.
7. Discard the supernatant.NOTE5) Add 800μl of 70% ethanol.
8. Centrifuge at 15,000 rpm for 10 min, at 4℃.
9. Dispcard supernatant. Dry the pellet.NOTE6)
10. Add 50～100μl TE or nuclease-free waterNOTE7). Dissolve the pellet to serve DNA as template for PCR.

* For Note1)～7)., see NOTES on page 5

NOTES

1) Add prepared additives onto *DNAs-ici!-P* 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.

3) Too much amount of starting materials may cause low DNA yield and/or quality, leading to inhibition of PCR amplification.

4) 1,000 μl pipette tips with tip holes closed by burning over alcohol lamps/lighters are good enough as homogenizer. For some materials, homogenization may be easier in a half volume of buffer, and add remaining buffer after homogenization and mix well.

5) Be careful not to wash out DNA pellet.

6) Overdrying may cause difficult of dissolve DNA into TE or water.

7) Amount of TE or water should be changed according to property of materials (species, organ, tissue, or condition) and PCR conditions (reaction volume, polymerase or reaction program).

This protocol is devised for DNA extraction from 10-50mg materials.

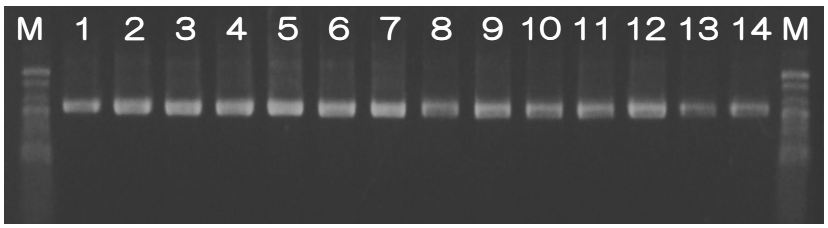
Troubleshooting Guide

Trouble	Suspected causes	Suggestions
Low DNA yield	Insufficient grinding and homogenization of materials.	Homogenize samples as thoroughly as possible.
	nsufficient DNA elution from materials into extraction buffer.	Homogenize the sample with *DNAs-ici!-P* and shake well, before moving on to the next procedure.
Yielding large amount of white precipitate after adding 2-propanol precipitation solution which remains even after washing out with 70% ethanol.	Large amount of protein and/or lipid content in samples.	Do step 3 and 4 of DNA extraction protocol over again and remove proteins and lipids

Examples

DNA extraction from polyphenol-rich lant tissues.

DNA was extracted using *DNAs-ici!-P* and PCR amplification was performed with obtained DNA as template using 18S rRNA gene detection primer pair(amplification size is 1,131 bp).



1.5% Agarose
M: Marker（100 base pair ladder）

1 basil（leaf）
2 japanese basil（leaf）
3 purple rice plant（leaf）
4 tomato（leaf）
5 apple fruit skin
6 nashi（pear) fruit skin
7 egg apple fruit skin
8 lotus（coat）
9 rosemary（leaf）
10 lavender（leaf）
11 gold crest（leaf）
12 ginkgo（leaf）
13 green tea
14 black rice（two grain）

（DNA was extracted from a tissue of approx. 50mg of each sample)

（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

*2-50fold dilution of obtained DNA was used.
**Stratagene Paq5000 DNA polymerase was used.

（cycling program）

95℃ 2 min.
94℃ 35 sec.
55℃ 30 sec.
72℃ 75 sec.
} 35 cycles
72℃ 7 min.

Product Line-up

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

For starch-rich samples ■*sDNAs-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 Rosaceae plant leaves with viscous substance ■*DNAs-ici!-R* DS-0003N (110 extractions) *Apples, pears, peaches, strawberries(leaves/petals/fruits)*	**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) *Woods, 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 mucosas and tissues of fishes** ■*DNAs-ici!-F* DS-0005 (210 extractions) *Various types of mucosas/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 Rosaceae 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 mucosas and tissues of fishes** ■*RNAs-ici!-F* RS-0005 (50 extractions) *Body surface mucosal cells, gene expression studies of epizoic microbes…etc.*

Contacts

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