DNAs-ici!-W

DS-0009

~DNA extraction buffer~

For woods/ dried plant tissues

User Manual

Ver. 1.0

RIZO Inc.

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Key Features

DNAs-ici!-W is a specialized DNA extraction buffer ideal for dried plant tissues such as wood.

DNAs-ici!-W provides speedy, low-cost and safe DNA extraction without spin-column purification.

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

*DNA may not be extracted depending on conditions of samples.

Kit Components

DNAs-ici!-W extraction buffer 85 mL
(for 110 extractions)
Additives A (powder) 2 bottles
Additives B (solution) 10 mL×1 bottle
Extraction adjuvants 5 mL × 2bottles

Expiry and storage conditions

Storage Condition:

■Store the buffer, Additive A/B and its solution refrigerated at 4℃.

■Store extraction adjuvants at room temperature(20-25℃) *.

Expiry:

■DNA extraction buffer/extraction adjuvants:
---6 months after opening

■Additives A/B: ---1 month after mixing*.

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

Ingredients may precipitate at low temperature(approx. 15℃ or below) in winter. In such case, incubate the buffer at 30-40℃ and make sure to dissolve the precipitates before use thoroughly. Precipitation does not affect the quality of the product.

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 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

Microcentrifuge (with rotor for 2ml tubes)

Others

1.5 ml tube

Micro pipettes（1,000 μl, 200 μl）

Pipette tips

Before use

Preparing additives

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

1. Put 10-50 mg[note1)] of samples into a clean tube (1.5ml)

2. Add 720 μl of *DNAs-ici!-W,* 80 μl of additives and 80 μl of extraction adjuvants to the 1.5ml tube above. Mix well [note2)].

3. Incubate [note3)] for 30 min at 65℃ and leave still for 5-10 min at room temperature [note4)]. (20-25℃)

4. Add 500 μl of phenol:chloroform (1 : 1,v/v). Mix well[5)].

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

6. Transfer 500 μl of supernatant to a clean tube(1.5 ml). Add 500 μl of (equal volume) 2-propanol. Mix well.

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

8. Discard supernatant[6)]. Add 1000 μl of 70% ethanol to wash pellet.

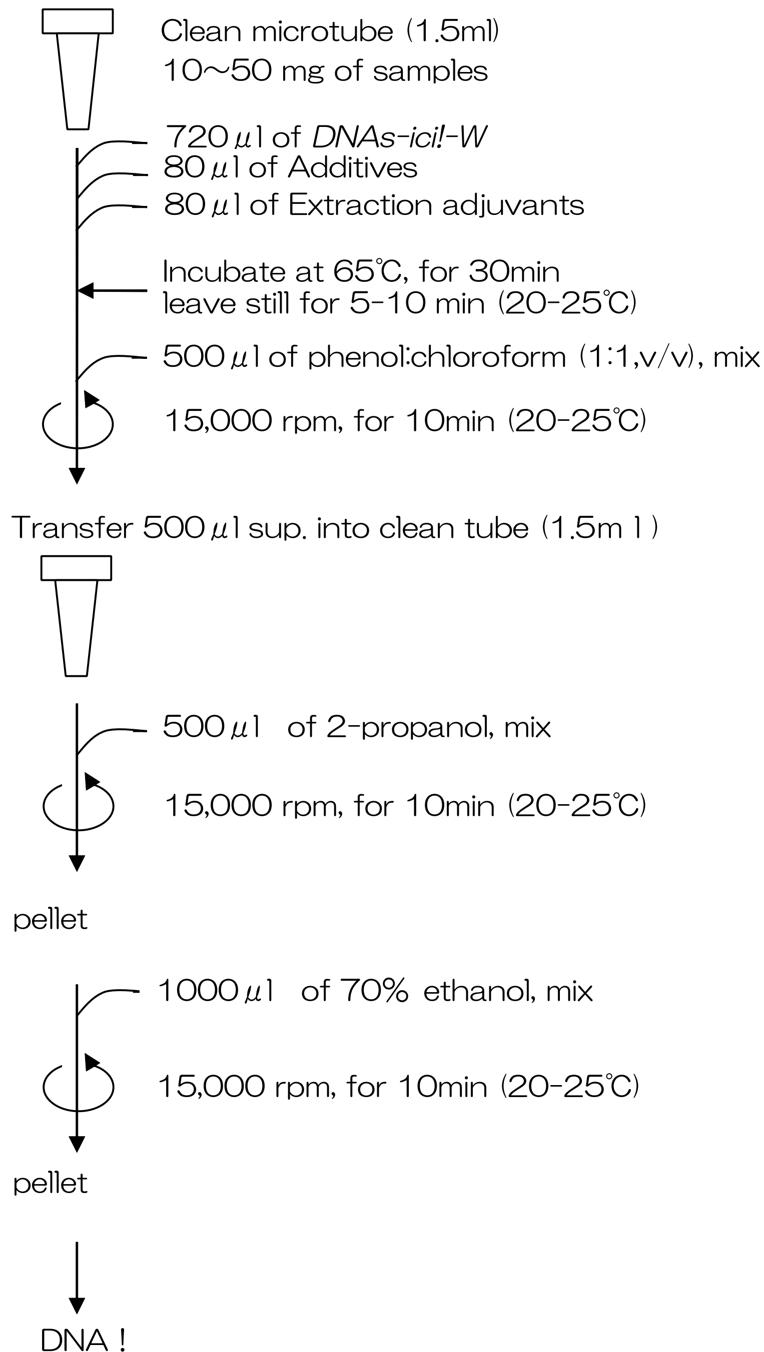
9. Centrifuge at 15,000 rpm for 10 min at room temperature(20-25℃).

10. Discard supernatant. Dry pellet.[7)]

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

*** For 1)-8), see NOTES on page 6**

DNA Extraction Protocol [Flow Chart]



NOTES

1) Cut plant tissues into small pieces with box cutters or scissors. Extraction efficiency may improve with smaller samples. (Ideal state for wood samples: wood chips which appear when sharpening pencils with box cutters/ sawdust which appear when sawing wood with saws)
2) Add additives and extraction adjuvants to *DNAs-ici!-W* immediately before use. Prepare additives solution per sample. Keep on going with the procedure even if white precipitates are observed when extraction adjuvants are added to *DNAs-ici!-W*.
3) Flopping the tube upside down during incubation process (approx. 15 min after starting incubation) makes it more effective.
4) DANGER!!! Allowing heat to radiate. Proceeding to step 4 immediately after incubation may result in leaking of phenol:chloroform (1 : 1,v/v) liquid from the crevice between the tube and tube cap.
5) Use rubber gloves, etc.
6) Be careful not to wash out DNA pellet.
7) Overdrying may make DNA's dissolution into TE or water difficult.
8) Optimal amount of TE or water varies according to the properties of materials (species, organs, tissues, or conditions) and PCR conditions (reaction volume, polymerase or reaction program). Adjust the amount of reagents according to the conditions above.

Troubleshooting

Trouble	Possible causes	Suggestions
Low DNA yield	Insufficient DNA elution from samples into extraction buffer.	In case of hard materials such as wood, use box cutters to scrape them into thin and small pieces. Wood-chip state is ideal.
		In case of relatively soft materials such as rush, use scissors to cut them into pieces of 2-3mm size. To further increase DNA yield, homogenize the sample with approx. 400 μl of extraction buffer, using1000 μl pipette tips*. Add each reagent to implement the buffer composition described in the protocol, and mix well before proceeding to the following steps. *Pipette tips with tip holes closed by burning over alcohol lamps/lighters are useful
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 samples.	Do step 4 and 5 of DNA extraction protocol over again and remove proteins and lipids

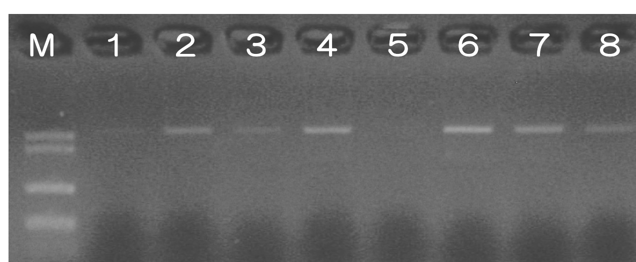
Examples

■ DNA extraction from wood and other dried plant tissues

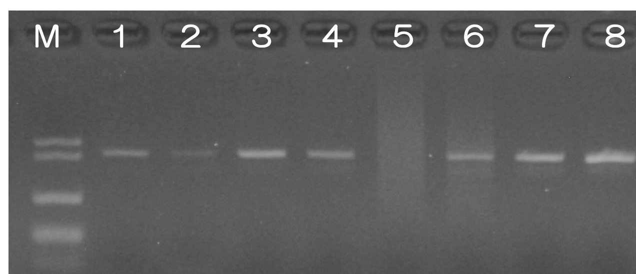
Using *DNAs-ici!-W,* DNA was extracted from wood and other dried plant tissues. Then, PCR amplification was performed with obtained DNA as templates, using three kinds of 18S rRNA gene detection primer pair which vary in the length of amplification products (amplification sizes are 500 bp, 400 bp, 214 bp).

（PCR Amplification Result）

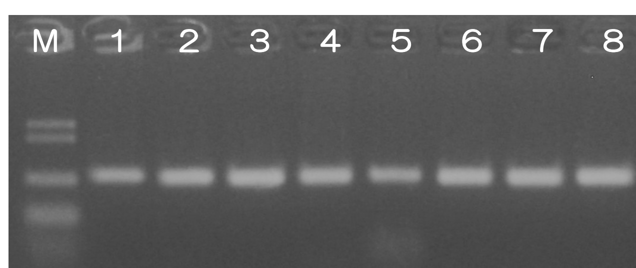
＜18S rRNA gene 500 bp＞



＜18S rRNA gene 400 bp＞



＜18S rRNA gene 214 bp＞



3.0% Agarose
M: Marker（from the top: 500 bp, 400 bp, 200 bp, 100 bp）

1. red meranti（wood）
2. wooden chopsticks
3. bamboo chopsticks
4. pencil
5. toothpick
6. chaff
7. rush
8. green tea leaf

*DNA was extracted from approx. 30mg of samples for 1.～7., 10mg of samples for 8. Samples except 8. are cut into small pieces by scraping with box cutters.

PCR amplification was performed using three kinds of 18S rRNA gene detection primer pairs which vary in the length of amplification products (amplification sizes are 500 bp, 400 bp, 214 bp）. With 500bp primer pair, depending on the sample, there were cases where no amplification was observed, however, with 214bp primer pair, all sample cases showed amplification＊

＊Sample dependent processing degree or condition leads to different resolution degree of genomic DNA. Amplification in each primer set might have been influenced by the fact above. Primer sets with amplification products of shorter length showed better amplification efficiency in the above experiment.

(Composition of reaction)

Template DNA	20 ng
1×Buffer	
dNTPs	0.2 mM
primer	0.5 μM
Taq polymerase*	0.6U
Total	15 μl

*Stratagene Paq5000 was used

(cycling program)

95℃ 2 min.
94℃ 30 sec.
55℃ 30 sec. } 35 cycles
72℃ 60 sec.
72℃ 7 min.

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