DNAs-ici!-S

DS-0001N

~DNA extraction buffer~

for 'starch-rich' plant materials and processed foods

User Manual

Ver. 1.0

RIZO Inc.

Table of Contents

Key Featues

DNAs-ici!-S is a specialized DNA extraction buffer ideal for 'starch-rich' plant materials, such as cereal grains, vegetative organs and their processed foods*.

DNAs-ici!-S provides speedy, low-cost and safe DNA extraction, without purification via spin-column or 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!-S DNA extraction buffer　85 mL x 2 bottles
(for 420 extractions)

Storage condition

Store the buffer refrigerated at 4℃.

Safety Warnings and Precautions

The use of this product is for research purpose only, not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

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

Reagents and Equipment Required

Reagents

2-propanol

70% Ethanol*

TE （10 mM Tris-HCl, 1 mM EDTA, pH 8.0） or

Nucease-free water

*Ethanol（molecular biology grade）：nuclease-free water =7：3(vol:vol)

Equipment

Microcentrifuge (with rotor for 2ml tubes)

Equipment for disruption and homogenization

Protocol For DNA Extraction

1. Prepare 400 μl of *DNAs-ici!-S* buffer in a microcentrifuge tube.

2. Add 30-60mg[1)] of ground samples[2)] into *DNAs-ici!-S* buffer prepared in step 1. Mix well by vortexing for 1 min.

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

4. Transfer 200 μl of supernatant into a clean microcentrifuge tube. Add 200 μl of (equal volume) 2-propanol. Mix well.

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

6. Discard the supernatant[3)]. Add 800 μl of 70% ethanol.

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

8. Dispcard the supernatant. Dry pellet.[4)]

9. Add 50～100 μl of TE or nuclease-free water[5)]. Dissolve the pellet. Serve DNA as a template for PCR.

*** For 1)-5)., see NOTES on page 5**

NOTES

1) Hard materials such as grains can be wrapped in aluminium foil and ground with pliers.
Soft materials can be directly added into *DNAs-ici!-S* buffer and crushed using hand homogenizers or micropipette tips.

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

3) Be careful not to wash out DNA pellet.

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

5) 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).

Technical Tips

In case of PCR amplification failure possibly caused by residuals in DNA, please consider trying A or B below.

A: After step 3, add equal volume of phenol/chloroform/isoamylalchol (SIGMA Cat. No. P2069 or equivalents) onto supernatant and vortex well. Centrifuge at 15000rpm for 10 min at room temperature(20-25℃).
Transfer the supernatant to a clean microtube, and then go back to step 4.

B: After step 2, incubate the mixture of sample and buffer at 65℃ for 30 min, then go back to step 4.
(Not recommended for materials which can be gelatinized by heating.)

This protocol is devised for DNA extraction from 30-60mg of materials.

Troubleshooting

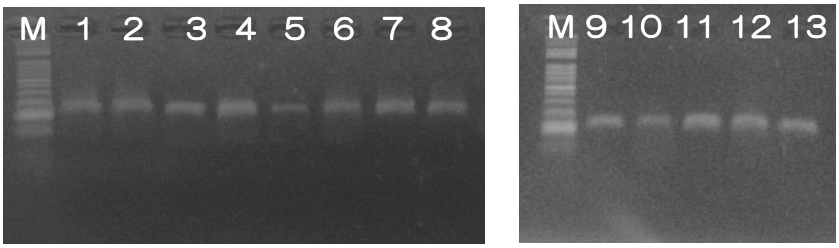
Trouble	Possible causes	Suggestions
Low DNA yield	Insufficient grinding and homogenization of materials.	Grind materials as fine as possible before adding into the extraction buffer. In case it's difficult to grind fine, homogenize materials in the buffer thoroughly using pestles or pipette tips.
	Insufficient DNA elution from materials into extraction buffer.	Vortex well after additing into the extraction buffer.
Yielding large amount of white precipitate after adding isopropanol which remains even after washing	Too much protein and/or lipid content in starting materials.	Add equal amount of phenol/chloroform/iso amylalchol （e.g. SIGMA Cat. No. P2069 or equivakents） to the supernatant of step 3 andvortex. Then centrifuge at 15,000rpm for 10min at room temperature(20～25℃).Transfer the supernatant into a clean collection tube. Then go back to step 4 and do subsequent procedures .

Examples

① DNA extraction from cereal seeds(raw or processed).

DNA of materials listed below were extracted using *DNAs-ici!-S* buffer.

PCR amplification was performed with obtained DNA as template and CP03 primers for plant DNA detection (amplification size is 124bp) listed in official method by Ministry of Health Labor and Welfare.



2% Agarose M: Marker（100 base pair ladder）

1 polished rice （one grain）
2 brown rice （one grain）
3 soy bean
4 azuki bean
5 wheat flour
6 starch powder
7 potato (raw)
8 sweet potato (raw)
9 buckwheat or 'soba' (processed)
10 mung bean (processed)
11 coan (processed)
12 oat (processed)
13 cooked rice (one grain)

（composition of reaction）

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

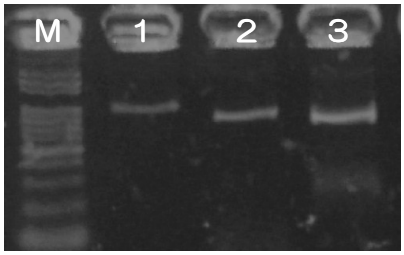
*10-100fold dilution of obtained DNA was used.
**Stratagene Pag5000 DNA polymerase was used.

（cycling program）

95℃ 2 min.
94℃ 30 sec.
60℃ 30 sec. } 40 cycles
72℃ 30 sec.
72℃ 7 min.

② PCR amplification test using DNA extracted from brown rice.

PCR amplification was performed using primers designed on various loci on rice chromosomes, using DNA of brown rice extracted by this product as template.



2% Agarose
M: Marker（100 base pair ladder）

1 Nucleotide pyrophosphatase precursor, coded on chromosome 1（900 bp）
2 Aminophospholipid fipase 10, coded on chromosome 5（825 bp）
3 UDP-glucose 6-dehydrogenase, coded on chromosome 12（809 bp）

（composition of reaction）

Template DNA*	1 （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

*10-100fold dilution of obtained DNA was used.
**Stratagene Paq5000 DNA polymerase was used.

（cycling program）

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

Contacts
RIZO Inc.
Amakubo 2-9-2 Tsukuba, Ibaraki, JAPAN.
Tel ; +81-29-852-9351
E-mail: info@rizo.co.jp
URL: http://www.rizo.co.jp/