DNAs-ici!-PF

DS-0007

~DNA extraction buffer~

For processed foods made from cereals

User manual

Ver. 1.0

RIZO Inc.

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Key Features

DNAs-ici!-PF is a specialized DNA extraction buffer best suited for various kind of processed foods mainly made from cereals*.
Without purification through spin-column, *DNAs-ici!-PF* provides speedy and low-cost DNA extraction.
Obtained DNA can be directly used for PCR as template.

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

Kit Components

DNAs-ici!-PF DNA extraction buffer 85 mL (for 140 extractions)

Storage Conditions

Store the buffer refrigerated at 4℃.
Use within 12 months of first opening the pack.

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, speculation and prices of this product are subject to change without notice.

Reagent and Equipment Required

Reagents

Phenol：chloroform（1：1, v/v）*
2-Propanol (molecular biology grade)
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 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)
Equipment for disruption and homogenization

Others

1.5 ml tube
Micro pipettes
Pipette tips

Protocol for DNA Extraction

1. Put 25-50mg[1)] powdered samples[2)] into a microcentrifuge tube.

2. Add 600 μl of *DNAs-ici!-PF* buffer. Mix by vortexing for 1 min.

3. Add 600 μl of phenol:chloroform（1：1,v/v）. Mix well by inversion.

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

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

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

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

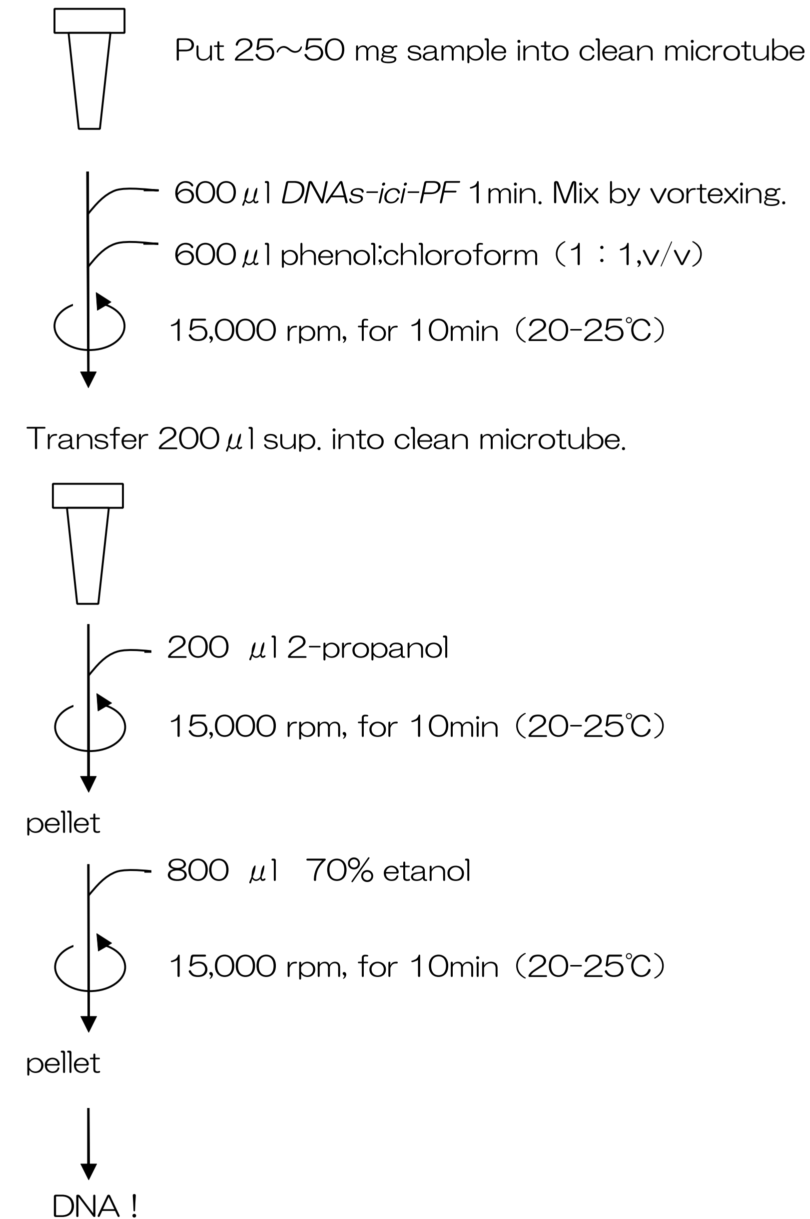
8. Centrifuge for 10 min at 15,000 rpm, at room temperature (20～25℃).

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

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

＊For 1)-5)., see NOTES on page 6

Protocol for DNA Extraction (Flow chart)



Notes

1) Uniformize sample well if necessary before extracting DNA.

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 cause difficulty of 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 to add a step below.

Mix equal amount of phenol/chloroform/isoamylalchol （e.g. SIGMA Cat. No. P2069） to supernatant of step 4, voltex, and centrifuge 15,000rpm for 10min at r.t. Transfer supernatant into a new collection tube. Follow step 5 and the rest.

This protocol is established for DNA extraction from 25-50mg 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.
	Insufficient DNA elution from materials into extraction buffer.	Vortex well after additing into the extraction buffer.
Yielding large amount of white precipitate after adding 2-propanol, which remains even after washing out with 70% ethanol.	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 4 and voltex. Then centrifuge at 15,000rpm for 10min at r.t.(20-25℃). Transfer the supernatant into a clean collestion tube. Then go back to step 5 and do subsequent procedures.

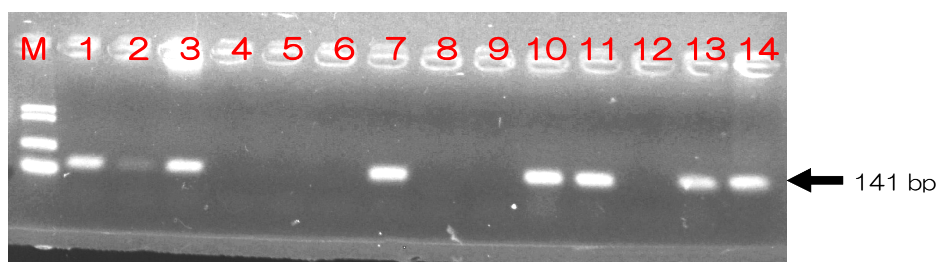
Examples

PCR detection of 'wheat' in processed foods

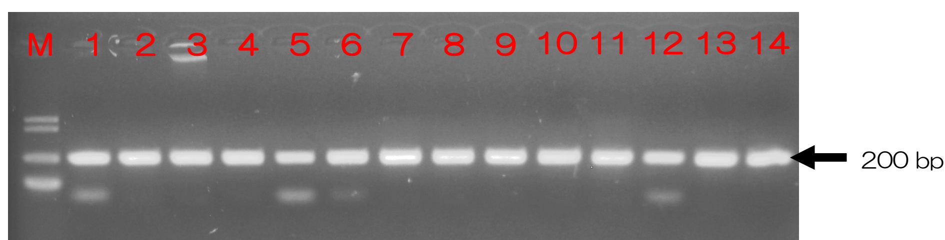
DNA of materials listed below were extracted using *DNAs-ici!-PF buffer.*

PCR amplification was performed with obtained DNA as template and primers for wheat DNA detection (amplication size is 141 bp) listed in official method by Ministry of Health Labor and Welfare. PCR amplification for positive control was performed with obtained DNA as template using 18S rRNA gene detection primer pair(amplification size is 200 bp).

primers for wheat DNA detection



primers for 18S rRNA gene detection (positive control)



3% Agarose
M: Marker（上から：500 bp、400 bp、200 bp、100 bp)

1. Tempura batter
2. Dry gluten
3. tortilla chips
4. Shrimp cracker
5. Ouster sauce
6. Okonomiyaki mix
7. Korean spicy miso
8. White rice malt miso
9. Freeze-dried bean curd
10. Bread crumb（dry）
11. Soba（dry）
12. Rice paper
13. Pasta（dry）
14. Wheatnoodle (udon)

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