DNAs-ici!-E

DS-0008

~DNA extraction buffer~

For soil, sludge, and environmental water

User Manual

Ver. 1.0

RIZO Inc.

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Key Features

DNAs-ici!-E is a specialized DNA extraction buffer ideal for various environmental materials such as soil, activated sludge and water.
DNAs-ici!-E provides speedy, low-cost and safe DNA extraction without spin-column purification.
Obtained DNA can be used in a variety of downstream applications.

Kit Components

DNAs-ici!-E extraction buffer　90mL
(for 100 extractions)

Storage conditions

Avoid heat and humidity. Store in a dark place at room temperature. Lower temperature may cause white precipitates to come out of the buffer. In such case, incubate the buffer at 28℃～30℃ and make sure to dissolve the precipitates before use thoroughly. Precipitation does not affect the quality of the product.

Expiry: 6 months after opening.

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, specifications and prices are subject to change without notice.

Reagents and Equipment required

Reagents

2-propanol

70% Ethanol*

phenol：chloroform（1 ： 1, v/v）**

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)

*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) are good as well. (e.g. SIGMA’ s Cat.No. P2069)

Equipment

Microcentrifuge (with rotor for 2ml tubes)

Others

1.5 ml tube

Micro pipettes

Pipette tips

Protocol for DNA Extraction

1. Put 900 μl of *DNAs-ici!-E* into a clean tube (1.5ml) (500 μl for liquid samples).

2. Add approx. 100 μl (or 50～100mg) of soil sample (500 μl for liquid samples) to 1. and shake vigorously to mix.

3. Mix well by tipping 2. with pestles or pipette tips. Be careful not to warm the sample above room temperature.[Note1)] (20～25℃)

4. Centrifuge at 15,000 rpm for 10 min at room temperature(20～25℃). Transfer 700 μl of supernatant into a clean tube.[note2)]

5. Add 300 μl of phenol:chloroform (1 : 1,v/v). Mix well.

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

7. Transfer 500 μl of supernatant into a fresh microtube. Add 500 μl (equal volume) of 2-propanol. Mix well.

8. Cool at -20℃ for 20 min.

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

10. Discard supernatant[3)]. Add 400 μl of 70% ethanol to wash pellet.

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

12. Discard supernatant. Allow pellet to air dry.[Note4)]

13. Dissolve pellet in 30 μl of TE or nuclease-free water[Note5)]. Serve contained DNA as a template for PCR.

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

NOTES

1) Extraction efficiency may improve by using bead-beater homogenizer. Excessive physical crash may result in degrading of DNA into smaller molecules.

2) In case of leaf soil samples, supernatant may contain floating substances, however, they will be removed in the following steps.

3) Be careful not to wash out DNA pellet.

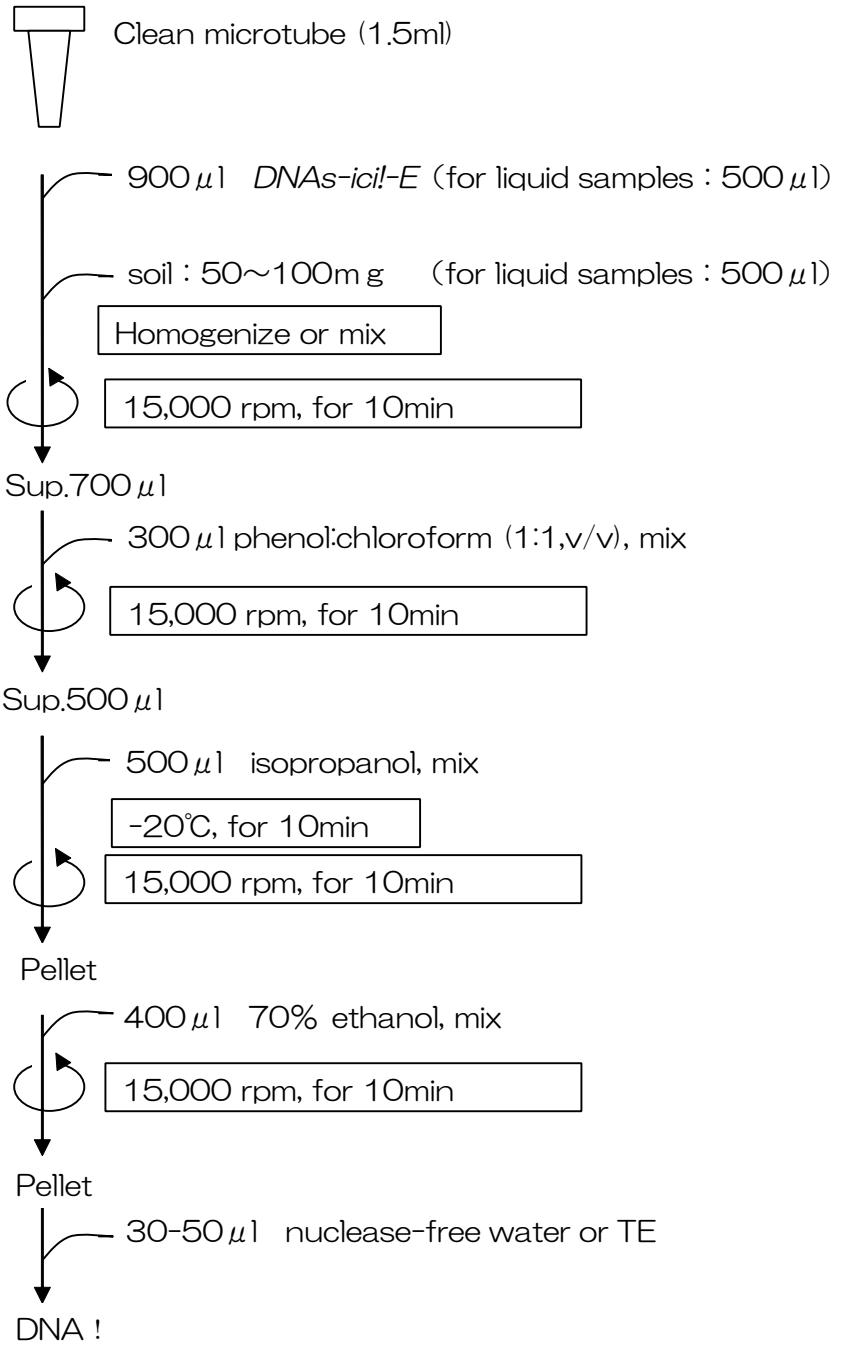
4) Overdrying may make DNA's dissolution into water difficult.

5) Optimal amount of TE or water varies according to the properties of materials and downstream application.

Technical Tips

This protocol is devised for DNA extraction from 30-60mg samples. Scaling up is possible for larger amount of samples.

Protocol for DNA Extraction (Flowchart)



Troubleshooting

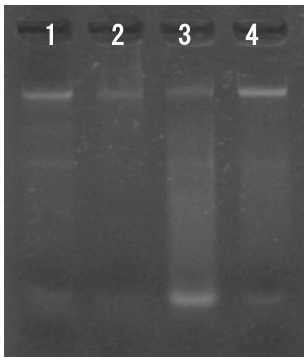
Trouble	Possible causes	Suggestions
Low DNA yield	Insufficient amount of biological cells in samples	Use as fresh soils as possible. Extract DNA from increased amount of samples, adopting scaled-up protocols. (Extraction may be difficult if very little DNA is contained in the sample)
	DNA pellets thrown out	DNA pellets are attached to the tube walls near the bottom. Visually checking, discard the supernatant carefully not to throw DNA pellets out.
DNA pellets or solutions being colored	Much corrosion acid contained in the sample	Mingling of corrosion acid into samples may be inhibited by performing the extraction process on ice. Extracted DNA can be refined with "Corrosion acid removal kit"* commercially available.
PCR amplification not going well(DNA extraction confirmed on electrophoresis or absorbance).	Corrosion acid remaining in DNA	Perform PCR amplification with DNA serially diluted to around 1000 fold. Or use "Corrosion acid removal kit"* commercially available.
Crystal precipitation observed on *DNAs-ici!-E*	Reagents kept at low temperature	Dissolve crystals thoroughly by leaving the reagents at room temperature (or warming in water-bath at 40℃) before use. No problem with quality and performance.

*PowerClean DNA Clean-up Kit (Funakoshi Co. Ltd. …etc.)

Examples

■DNA extraction from soil

Using *DNAs-ici!-E,* DNA was extracted from soil of artificial rice-field(volcanic-ash rich), garden soil(leaf-soil rich), and two kinds of activated sludge according to the protocol. Then, 1/3 of obtained DNA solution was electrophoresed.



1 : soil of artificial rice-field

2 : garden soil

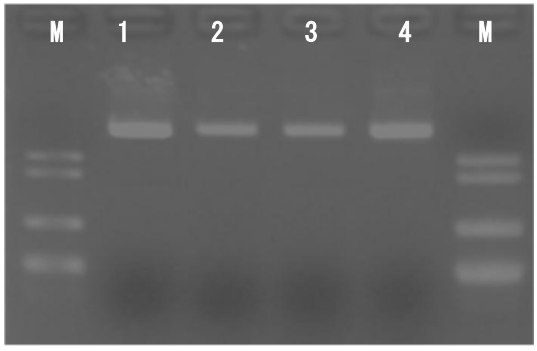
3 : activated sludge A (liquid-waste disposal from a source/mustard factory)

4 : activated sludge B (liquid-waste disposal from a school-meal facility)

0.8% Agarose /0.5×TBE

High molecular weight genomic DNA was extracted from soils of all above(1 ～ 4: volcanic-ash rich, leaf-soil rich, and two kinds of activated sludge).

Subsequently, PCR amplification was performed with obtained DNA as templates using primers developed on 18S rRNA gene for bacteria (amplification size: 732bp). 1 μl of extracted DNA diluted to 250 fold was used as templates.



M : Size markers
(100, 200, 400, 500bp)

1 : rice-field soil

2 : garden soil

3 : activated sludge A

4 : activated sludge B

PCR amplification was performed well for soils of all above(1～4: rice-field soil, garden soil, and two kinds of activated sludge).

Reference for primers : Kuske et al., *Appl Environ Microbiol.* 64(7):2463-72(1998))

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