DNAs-ici!-F

DS-0005

~DNA extraction buffer~

for mucous membranes and tissues of fishes

User Manual

Ver. 1.01

RIZO Inc.

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Key Features

DNAs-ici!-F is a specialized DNA extraction buffer ideal for mucous membranes and tissues of fishes.

Samples for DNA extraction can be obtained by gently rubbing the body surface of living fish using swabs without wounding fish bodies.

DNAs-ici!-F is also suitable for DNA extraction from fins, muscles, internal organs, saliva, meats, dried or processed seafood and meats*.

DNAs-ici!-F provides speedy, low-cost DNA extraction with a small number of handling steps.

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

*DNA may not be extracted depending on sample conditions.

Kit Components

DNAs-ici!-F 85 mL

(for 210 extractions)

Storage Conditions

Store the buffer at 4℃ after opening.

Before use, warm the buffer to room temperature to resolve crystals when crystals observed.

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. (i.e. SIGMA’s Cat.No. P2069)

Equipment

Microcentrifuge (with rotor for 2ml tubes)
Heating apparatus (heating block, water bath, etc.)

Others

1.5 ml tube
Micro pipettes
Pipette tips
Swabs（individually packaged）
Stout scissors（cooking scissors or nipper）

Protocol For DNA Extraction

1. Put 400 μl of *DNAs-ici-F* into a clean tube (1.5ml).

2. Using swabs, gently rub over the body surface of fish[NOTE1)]. In case of tissue samples such as fins, gills, internal organs or muscles, collect samples as much as 5～50mg.

3. Put swabs of step2 into tubes of step1 and blend with *DNAs-ici-F*. Cut the swab stick off with stout scissor and cap the tube. In case of tissue samples, put samples into tubes of step1 and blend with *DNAs-ici-F* [NOTE2)].

4. Incubate tubes at 60℃ for 15 min, using heating apparatus.

5. Put tubes on ice to chill. Add 200 μl of phenol: chloroform(1:1,v/v), mix well by inversion. No need to remove swabs (this step can be skipped for DNA extraction from swabs).

6. Centrifuge at 15,000 rpm for 10 min, at room temperature.

7. Transfer 200 μl of supernatant to a clean tube (1.5 ml) and add 200 μl of 2-propanol. Mix well.

8. Centrifuge at 15,000 rpm for 10 min, at room temperature.

9. Discard the supernatant[NOTE3)]. Add 400 μl of 70% ethanol to wash out the pellet.

10. Centrifuge at 15,000 rpm for 10 min, at room temperature.

11. Discard the supernatant. Allow the pellet[NOTE4)] to air dry.

12. Dissolve the pellet in 50～100 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) Take the sample fish out of the water quietly using bare hands or soft net and work quickly. In case the sample fish is mingled with other fishes in the pool, put the sample fish into clean water to allow it swim alone for a while before collecting tissue samples to prevent contamination.

2) Mash the sample with a pestle, as necessary.

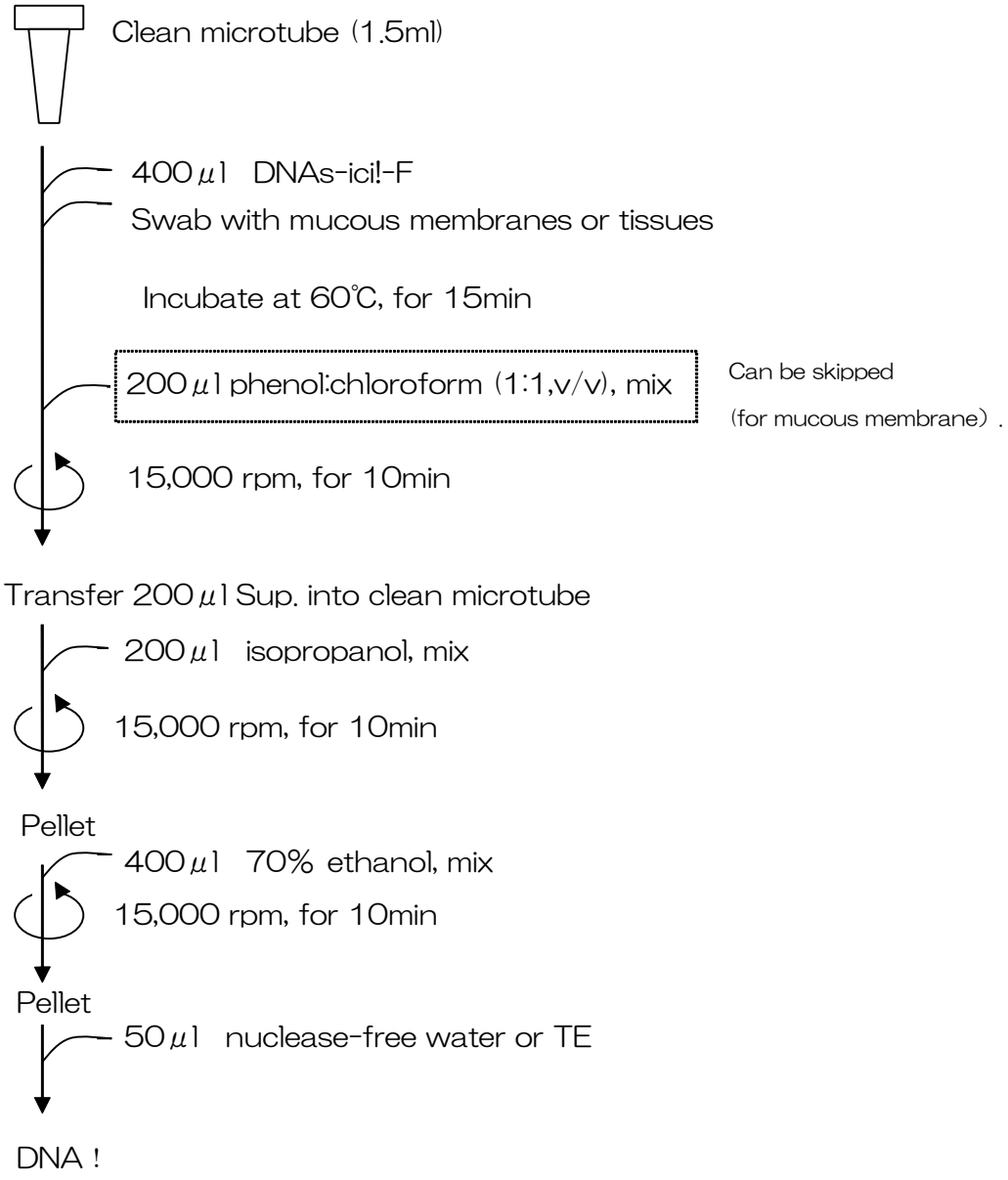
3) Be careful not to wash out DNA pellet.

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

5) Amount of TE or water varies according to properties of samples (species, organs, tissues, or conditions) and downstream research application.

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

DNA Extraction Protocol [Flow Chart]



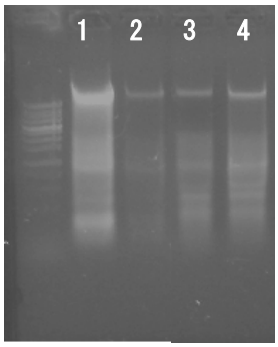
Troubleshooting

Trouble	Possible causes	Suggestions
Low DNA yield	Insufficient amount of samples.	Collect body surface mucosa to twist around the entire swab.
	Degradation of DNA before extraction.	Put swabs into *DNAs-ici!-F* immediately after sampling. In case of tissue samples, DNAs can be degraded before extraction procedure depending on storage conditions .
Many contaminants contained in DNA	Too much amount of starting samples.	Reduce the amount of starting samples to 1～10mg.
	Extremely protein-rich samples.	If much protein found in obtained DNA, add *DNAs-ici!-F* into DNA solution to make it 400 μl and repeat Step5～.

Examples

① DNA extraction from body surface mucosa and tissues of freshwater fish(goldfish).

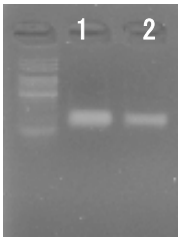
DNA was extracted from body surface mucosa, gill, kidney, and skeletal muscle of gold fish using *DNAs-ici!-F*.



1 : gold fish (body surface mucosa)
2 : gold fish (gill)
3 : gold fish (kidney)
4 : gold fish (skeletal muscle)
A swabful of DNA from body surface mucosa and 20mg of DNA from other tissues each was extracted in accordance with the protocol. And then, 5μl each was electrophoresed.

② DNA extraction from body surface mucosa and fins of freshwater fish(goldfish) and PCR amplification

DNA was extracted from body surface mucosa and fins(ventral fin 2mg) of goldfish using *DNAs-ici!-F.* And, PCR amplification was performed with the obtained DNA as templates using primers developed on 18S rRNA gene for freshwater fish(amplification size: 151bp).



【Templates】
1 : gold fish (body surface mucosa)
1 μl of obtained DNA
2 : gold fish (fin)
1 μl of obtained DNA

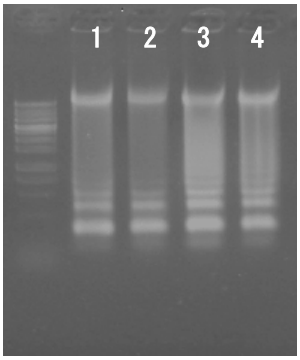
PCR was performed further using 1 μl of diluted DNA(10f,100f) as templates.

Upper1 : gold fish (body surface mucosa)
1 μl of obtained DNA(10f dilution)
Upper2 : gold fish (fin)
1 μl of obtained DNA(10f dilution)
Lower1 : gold fish (body surface mucosa)
1 μl of obtained DNA(100f dilution)
Lower2 : gold fish (fin)
1 μl of obtained DNA(100f dilution)

Confirmed amount of DNA obtained:
Body surface mucosa: amount for PCR 5000 times or more
Fin: amount for PCR 500 times or more

③ Use as a "Temporary Storage Solution" after sampling

Put a sample(mucosa of freshwater fish(goldfish))-carrying swab into a tube(1.5ml) together with *DNAs-ici!-F* and cap the tube. DNA extraction was performed after storage at room temperature(２５℃～３０℃) for 4 days and 11 days each.



1 : after 4 days at room temperature
2 : after 11 days at room temperature
3 : after 4 days cold storage
4 : after 11 days cold storage
DNA was extracted in accordance with the protocol, and 5 μl each was electrophoresed.

DNA was extracted without degradation even after 11days storage at room temperature.

DNAs-ici!-F is useful as a temporary storage solution on field work during such DNA extraction can't be performed immediately after sample collection.

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