DNAs-ici-VS

DS-0004

~DNA extraction buffer~

for plant materials containing viscous substance

User Manual

Ver. 1.0

RIZO Inc.

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Key Featues

DNAs-ici!-VS is a specialized DNA extraction buffer ideal for agricultural products containing lots of viscous substance*, such as green onions, mekabu (thick wakame leaves) and nameko mushrooms which show high viscosity when homogenized.

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

Kit Components

DNAs-ici!-VS DNA extraction buffer 85 mL
(for 110 extractions)
Additives A (powder) 2 bottles
Additives B (solution) 10 mL×1 bottle
DNA precipitation solution*
*Please add 60ml of isopropanol before use.

Expiry and storage conditions

Storage Condition: Store the buffer refrigerated at 4℃.
Expiry:

Additives A/B: 1 month after mixing*.
DNA extraction buffer: 6 months after opening
DNA precipitation solution: 6 months after preparation
* Mixed additives can be frozen for long-term storage.

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 price of the product are subject to change without notice.

Reagents and Equipment Required

Reagents

2-propanol
Ethanol
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 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

High speed refrigerated micro centrifuge

Others

1.5 ml tube
Micro pipettes（1,000 μl, 200 μl）
Pipette tips

Before use

① Preparing additives

Put 5ml of additives B (blue label) into additives A (red label) and mix well*. Prepared additives lysate shall be added onto *DNAs-ici!-VS* immediately before extracting DNA.

*Prepared additives lysate expires1month after mixing. Please keep it refrigerated at 4℃ in the dark till it is used.

② Preparing DNA precipitation solution

Add 60ml of isopropanol onto the bottle of DNA precipitation solution and mix well. Keep the prepared solution refrigerated at 4℃ till it is used.

Protocol for DNA Extraction

Mix 720 μl of *DNAs-ici!-VS* with 80 μl of prepared additives[NOTE1)] per sample. Make this as many as number of samples.

1. Put 400μl of *DNAs-ici!-VS* with additives into a clean tube(1.5ml) [NOTE2)] and add 30-50mg of samples.[NOTE3)]
2. Homogenize samples using a microtube pestle. [NOTE4)].
3. Add a further 400 μl of *DNAs-ici!-VS* with additives. Mix well.
4. Add 500 μl of phenol:chloroform (1 : 1,v/v). Mix well.
5. Centrifuge at 15,000 rpm for 10 min, at room temperature(20～25℃).
6. Transfer 600 μl of supernatant to a clean tube(1.5 ml) and add 480 μl (0.8 × supernatant vol) of 99.5% ethanol. Mix well[NOTE5)].
7. Centrifuge at 3,000 rpm for 1 min, at room temperature(20～25℃).
8. Transfer 700 μl of supernatant to a clean tube(1.5 ml) without disturbing the pellet and add 700 μl (equal volume) of prepared DNA precipitation solution. Mix well.
9. Centrifuge at 15,000 rpm for 10 min, at room temperature(20～25℃).
10. Discard the supernatant[NOTE6)]. Add 1,000μl of 70% ethanol. Shake well to wash out the pellet.
11. Centrifuge at 15,000 rpm for 10 min, at 4℃.
12. Dispcard the supernatant. Dry the pellet. [NOTE7)]
13. Add 50～100μl of TE or nuclease-free water[NOTE8)]. Dissolve the pellet to serve DNA as a template for PCR.

＊ For 1)-8)., see NOTES on page 5

NOTES

1) Add prepared additives onto *DNAs-ici!-VS* immediately before use. Don't use those which elapsed a day or more after mixing.

2) In case of cryopreserved tissues, the sample needs to be dipped into extraction buffer before thawing.

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

4) 1,000 μl pipette tips with tip holes closed by burning over alcohol lamps/lighters are good enough as homogenizer.

5) Depending on the type of sample, 5 minutes incubation on the ice between step 6 and 7 may make the removal of viscous substance more effective. Be careful not to try this procedure for samples with no viscous substance, which may lead to DNA yield reduction.

6) Be careful not to wash out DNA pellet.

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

8) 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).

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

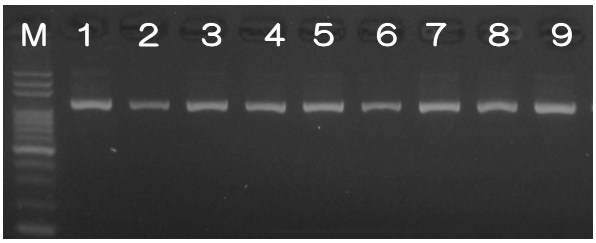
Troubleshooting

Trouble	Possible causes	Suggestions
Low DNA yield	Insufficient grinding and homogenization of materials.	Homogenize samples as thoroughly as possible.
	Insufficient DNA elution from materials into extraction buffer.	Homogenize the sample with *DNAs-ici!-VS* and shake well, before moving on to the next procedure.
Yielding large amount of white precipitate after adding DNA precipitation solution which remains even after washing out with 70% ethanol.	Large amount of 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 agricultural products with viscous substance.

DNA was extracted using *DNAs-ici!-VS* and PCR amplification was performed with obtained DNA as template using 18S rRNA gene detection primer pair(amplification size is 1,146 bp).



1.5% Agarose
M: Marker
(100 base pair ladder)

1 green onion
2 satoimo (taro)
3 nameko mushrooms
4 mekabu(thick wakame)
5 rose (flower petal)
6 chrysanth(flower petal)
7 apple(flesh of fruit)
8 aloe
9 natto

(DNA was extracted from a tissue of approx. 50mg)

Composition of reaction mix

	(μl)
Template DNA*	1～3
10×Buffer	2
dNTP mixture (2.0mM each)	2
primer (4 pmol each/μl)	2
Taq** (5units/μl)	0.17
H_2O	
Total	20 μl

*2-100fold dilution of obtained DNA was used.
The optimal DNA dilutions vary by the type of samples and species.
**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.

Product Line-up

[DNA extraction buffer "*DNAs-ici!*" series]

For starch-rich samples ■*sDNAs-ici!-S* DS-0001N (420 extractions) *Rice(brown/polished), wheat, chestnuts, Japanese millets, beans, other grains, tubers and roots, cooked rice, flours…etc.*	**For plant tissues containing viscous substance** ■*DNAs-ici!-VS* DS-0004 (110 extractions) *Green onion, satoimo (taro), mekabu(thick wakame leaves), natto, aloe…etc.*
For Rosaceae plant leaves with viscous substance ■*DNAs-ici!-R* DS-0003N (110 extractions) *Apples, pears, peaches, strawberries(leaves/petals/fruits)*	**For environment-related materials such as soil and activated sludge** ■*DNAs-ici!-E* DS-0008 (100 extractions) *Soil including volcanic ash, leaf molds, activated sludge*
For lipid-rich plant seeds ■*DNAs-ici!-L* DS-0006 (180 extractions) *Peanuts, almonds, soy beans, walnuts, cashew nuts*	**For woods/dried plant tissues** ■*DNAs-ici!-W* DS-0009 (110 extractions) *Woods, bamboo products, straws, rushes, rice hulls…etc.*
For processed foods ■*DNAs-ici!-PF* DS-0007 (140 extractions) *Tempura Agedama, tortilla chips, Korean miso, Japanese miso, freeze-dried tofus, pasta, soba noodles, udon noodles…etc.*	**For body surface mucosas and tissues of fishes** ■*DNAs-ici!-F* DS-0005 (210 extractions) *Various types of mucosas/tissues, saliva, meats, dried seafood…etc.*

[RNA extraction buffer "*RNAs-ici!*" series]

For starch-rich samples ■*RNAs-ici!-S* RS-0001N (210 extractions) *Endosperm of cereal crops such as rice, wheat, beans (unripe/full-ripe). Vegetative reproduction organs. Roots.*	**For Rosaceae plant leaves with viscous substance** ■*RNAs-ici!-R* RS-0003N (150 extractions) *Apples, pears, peaches, strawberries and cherries (leaves/petals/fruits). Petals of chrysanthemums, welsh onions and taros.*
For polyphenol-rich samples ■*RNAs-ici!-P* RS-0002N (170 extractions) *Herbs, Angelica keiskeis, leaves of purple rice, black rice, arctium lappas, lotus roots…etc.*	**For body surface mucosas and tissues of fishes** ■*RNAs-ici!-F* RS-0005 (50 extractions) *Body surface mucosal cells, gene expression studies of epizoic microbes…etc.*

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