DNAs-ici!-L

DS-0006

~DNA extraction buffer~

For lipid-rich plant seeds

User Manual

Ver. 1.0

RIZO Inc.

Table of Contents

Key Featues

DNAs-ici!-L is a specialized DNA extraction buffer ideal for lipid-rich plant seeds such as sunflower seeds, peanuts, almond or processed nuts. Obtained DNA can be used in a variety of downstream applications.

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

Kit Components

DNAs-ici!-L DNA extraction buffer　80mL
(for 180 extractions)

＊Please add 10ml of ethanol before use.

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 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.

Reagents and Equipment Required

Reagents

2-propanol

99.5% 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

Heating apparatus (heating block, water bath, etc.)

Others

1.5 ml tube

Micro pipettes（1,000 μl、200 μl）

Pipette tips

Before use

1)Preparation of extraction buffer

Add 10ml 99.5% ethanol onto *DNAs-ici!-L* extraction buffer. Mix well. Store at 4℃ after preparation.(80ml).

2)Preparing additives

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

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

3) Preparing DNA precipitation solution

Add 60ml of 2-propanol onto the bottle of DNA precipitation solution and mix well. Keep the prepared solution refrigerated at 4℃ till it is used.

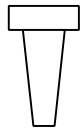
Protocol For DNA Extraction

Mix 450 μl of *DNAs-ici!-L* with 50 μl of prepared additives[NOTE1)] per sample. Make this as many as number of samples.

1. Put 500 μl of *DNAs-ici!-L* with additives into a clean tube(1.5ml) [NOTR2)] and add 30-50mg of samples.[NOTE3)]
2. Homogenize samples using a microtube pestle.[NOTE4)].
3. Incubate tubes at 65℃ for 10min,using heating apparatus.
4. Incubate 5min. at room temp.
5. Add 500 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 200 μl、of supernatant to a clean tube (1.5 ml) and add 500 μl of prepared DNA precipitation solution. Mix well.
8. Centrifuge at 15,000 rpm for 10 min, at room temperature.(20-25℃)
9. Discard the supernatant[NOTE5)]. Add 1 , 0 00 μl of 70% ethanol.
10. Centrifuge at 15,000 rpm for 10 min, at 4℃.
11. Dispcard the supernatant. Dry pellet.[NOTE6)]
12. Add 50 ～ 100 μl of TE or nuclease-free water.[NOTE7)] Dissolve the pellet. to Serve DNA as template for PCR.

＊For 1)-7）., see NOTES on page 7

DNA Extraction Protocol (Flow Chart)



Prepare 500 μl of extraction buffer with additives in a clean microtube (1.5m l)

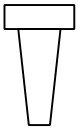
Put sample (30~50 mg) and pulverize

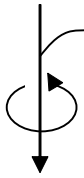
Incubated at 65℃, for 10min.
Cool at room temperature (20~25℃) for 5min.

500 μl phenol:chloroform (1 : 1,v/v) ,mix

15,000 rpm, for 10min (20-25℃)

Transfer 200 μl Sup. into clean microtube(1.5m l)

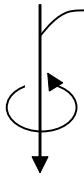




500 μl of prepared DNA precipitation solution.

15,000 rpm, for 10min (20-25℃)

Pellet



800 μl of 70% ethanol

15,000 rpm, for10min (20-25℃)

Pellet

DNA !

NOTES

1) Add prepared additives onto *DNAs-ici!-L* 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) Be careful not to wash out DNA pellet.

6) Overdrying may cause difficulty of dissolving DNA into TE or water.

7) 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 Guide

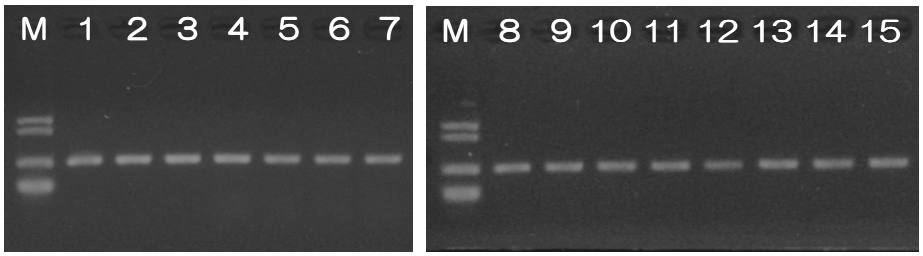
Trouble	Suspected causes	Suggestions
Low DNAyield	Insufficient grinding and homogenization of materials.	Homogenize samples as thoroughly as possible.
	Insufficient DNA elution from materials into extraction buffer.	Homogenize the sample with *DNAs-ici!-L* and shake well, before moving on to the next procedure.
Yielding large amount of white precipitate after adding DNA precipitation solution which remains even after washing out with 70% ethanol.	Too much protein and/or lipid content in starting materials.	Mix equal amount of phenol/chloroform/iso amylalchol （e.g. SIGMA Cat. No. P2069 or equivakents） to supernatant of step 6, voltex, and centrifuge 15,000rpm for 10min at r.t. Transfer supernatant into a new collection tube. Then back to step 7.

Examples

DNA extraction from lipid-rich plant seeds

DNA was extracted using *DNAs-ici!-L* and PCR amplification was performed with obtained DNA as template using 18S rRNA gene detection primer pair(amplification size is 200 bp).

*Universal primers designed to amplify 18SrRNA fragment from various plant DNA were used.



3.0% Agarose
M: Marker(500 bp、400 bp、200 bp、100 bp)

1 Earth nut pea
2 Soybean（seed）
3 Lentil（seed）
4 Avocado（seed）
5 Sunflower（seed）
6 Rape（seed）
7 Sesame
8 Roast almond （salty taste）
9 Roast peanuts （salty taste）
10 Roast pistachio （salty taste）
11 Roste macadamia nut （salty taste）
12 Roast walnut （salty taste）
13 Roast cashew nut （salty taste）
14 Peanut butter
15 Fried giant cone （salty taste）

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

（conmosition of reaction）

Buffer	1×
dNTPs	0.2 mM
$MgCl_2$	2 mM
Primer	0.5 μM
DNA	20 ng
Taq polymerase	0.6U
Total	15 μℓ

（cycling program）

95℃ 2 min.
94℃ 30 sec.
54℃ 30 sec.
72℃ 60 sec.
} 35 cycles
72℃ 7 min.

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