DNA extraction kit from plants, yeasts and bacteria

ISOPLANT

Manual (8th edition)

Code No. 314-02731 For 100 extractions

Code No. 310-02733 For 20 extractions

NIPPON GENE CO., LTD.

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I Product description

ISOPLANT is a kit for extracting DNA from plants, yeasts and bacteria in a short period of time. ISOPLANT disrupts cell walls, cell membranes, nuclear membranes and the like with benzyl chloride, which is the main component of the Solution II of the ISOPLANT. Since the sample can be solubilized in the presence of a surface-active agent, DNA can be extracted without grinding, especially in the case of plants. Therefore, this is very convenient when processing many samples. DNA can be extracted without pretreatment not only from dicot plants, but also monocot plants. The obtained DNA can be used for PCR and restriction enzyme reactions as-is.

II Contents of kit

			(For 100 extractions)	(For 20 extractions)
Solution I	:	Extraction Buffer*	30 ml	6 ml
Solution II	:	Lysis Buffer	15 ml	3 ml
Solution III	:	Sodium Acetate (pH 5.2)	15 ml	3 ml
TE	:	10 mM Tris-HCl (pH 8.0), 1 mM EDTA	10 ml	2 ml
RNase A	:	1 mg/ml	100 µl	20 µl

* White precipitates may appear in the Solution I but this does not affect the quality. In such cases, use the solution after completely dissolving the crystals in a water bath at about 37°C. Also, handle the solution with care because the Solution I contains a protein denaturant and the like. If the Solution I enters eye or contacts skin, immediately wash well with large amounts of water.

III Storage

Chilled storage (4°C)

All the reagents except RNase A can be stored at room temperature.
Further, when the kit is not to be used for a long period of time, store RNase A frozen (-20°C).

IV Precautions

○ This product is a reagent for research and cannot be used for other purposes such as for pharmaceutical purposes. Also, this product should be handled only by persons who have basic knowledge of reagents.

○ The main component of the Solution II included in this product is benzyl chloride. Benzyl chloride is a designated hazardous material under the Fire Service Act (group 4 hazardous material, group 2 petroleum, hazard degree III, flammable, keep away from flames). Handle with care. See the following for hazardous effects and handling precautions.

· Hazardous effects…

This product has extremely strong irritant properties and tearing properties to skin and mucous membranes, and may cause chemical burn on contact. Inhaling vapor at high concentrations may cause symptoms such as coughing, headaches, burning sensations, hypoesthesia, consciousness loss, tremor of limbs and paralysis.

· Handling precautions…

Keep away from flames and avoid contact with strong oxidants.

Install a local ventilating apparatus in the area where the reagent is handled, and close the lid of the container tightly after use.

Wear proper protective gears so as not to inhale or allow contact to eye, skin and clothing.

If the eye, skin or the like makes contact with the reagent, perform the following procedures.

If the reagent enters eye :

Immediately wash with a large amount of water for not less than 15 min.

Skin contact :

Wash with a large amount of water using soap.

Inhalation :

Rest and stay warm in a well-ventilated area.

Accidental ingestion :

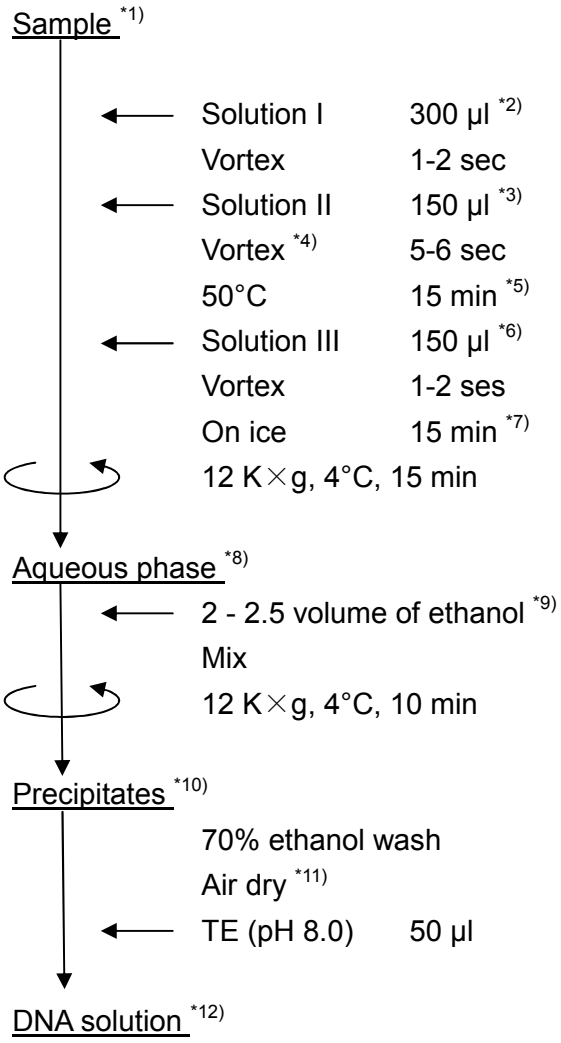
Rinse out the mouth, drink activated charcoal suspended in water and consult a physician. Since the mucous membrane in the stomach and the like is damaged, do not force vomiting.

Also, if there are other abnormal symptoms, immediately consult a physician.

For storage, avoid direct sunlight, keep in a cool dark place and avoid contact with air by hermetically sealing the product. Further, if a minute amount of a metal such as iron is mixed in the container, a strong degradation reaction may occur, causing break-up of the container.

○ Handle this product in accordance with the descriptions in the manual. We cannot take responsibility for problems caused if the product is not handled according to the descriptions in the manual.

V Protocol



*1) In case of plants, cut 0.01-0.1 g of the sample into 3-5 mm squares with scissors and then use. Although the volume may change depending on the variety of plant, it is preferable to immerse the sample entirely in the lower layer (Solution II) when Solution II is added.
Also, use fresh samples as much as possible. Even stored frozen, the recovery rate of DNA may be lower when the storage time is long.
In the case of yeast, bacteria and the like, use the precipitates (～0.03 g) obtained by centrifuging a 1.5 ml culture.

*2) Since Solution I contains SDS, white crystals may form when stored at a low temperature. In such a case, use Solution I after completely dissolving in a water bath at about 37°C.

*3) The main component of the Solution II included in this product is benzyl chloride. Benzyl chloride is a designated hazardous material under the Fire Service Act. Handle with care. (See p. 2, *IV Precautions.*)
If eye, skin or the like make contact with the reagent, perform the following procedures immediately.

If the reagent enters eye :
Immediately wash with a large amount of water for not less than 15 min.

Skin contact :
Wash with a large amount of water using soap.

Inhalation :
Rest and stay warm in a well-ventilated area.

Accidental ingestion :
Rinse out the mouth, drink activated charcoal suspended in water and consult a physician. Since the mucous membrane in the stomach and the like is damaged, do not force vomiting.

Also, if there are other abnormal symptoms, immediately consult a physician.

*4） White turbidity is formed by vortexing.

*5） Cell walls, cell membranes, nuclear membranes and the like are disrupted by benzyl chloride, which is the main component of the Solution II, and DNA is dissolved into aqueous phase (Solution I containing surface-active agent). For plant samples, the morphological changes are normally not visible.

*6） By adding Solution III, white turbidity is formed.

*7） White turbidity is further formed when cooled on ice.

*8） Avoid taking substances other than the aqueous phase, such as benzyl chloride in the organic phase, the solid white substance found after the centrifugation and floating pieces of the plant, as much as possible. If substances other than the aqueous phase are mixed in, centrifuge again and collect only the aqueous phase. In order to prevent physical cleavage of DNA., cut the pipette tips.

*9） In situations where PCR and restriction enzyme reactions do not proceed well, reactions may be improve by performing ethanol precipitation twice. When performing the ethanol precipitation, use ethanol cooled at -20°C, and centrifuge immediately after the addition. If the mixture is left standing at -20°C for a long period of time, this may cause co-precipitation of contaminants.

*10）DNA precipitates may be invisible. Depending on the variety of the sample, precipitates other than DNA may be formed. For example, rice plant may cause brown precipitates, and spinach may cause white precipitates which are different from DNA.

*11）Since completely dried precipitates become very difficult to dissolve, make sure not to dry too much.

*12）Add TE when non-DNA debris precipitates out (see *10)). If it does not dissolve completely by adding TE, let it stand on ice for a while, and collect the supernatant after brief centrifuge. Note that if precipitates are found to have brownish color, see VI troubleshooting in p.5. When RNA in the DNA solution is to be removed, add attached RNase A to a final concentration of 10-20 μg/ml and react at 37°C for 30 min. If necessary, perform phenol/chloroform treatment after the reaction.

VI Troubleshooting

Trouble	Countermeasure
White crystals appear in the Solution I.	Dissolve crystals completely in a water bath at about 37°C, stir so that the content becomes homogeneous and then use it.
DNA is brown after ethanol precipitation and centrifuging. Does it have any influence on PCR?	When a brownish color is found in precipitates, contamination by polyphenol, which is rich in plants, may be considered. This substance inhibits PCR, but the degree of inhibition may be lowered by adding 2-Mercapto-ethanol at 2% (final concentration) to Solution I beforehand.
When samples such as legume plants containing a large amount of sugar are used, gelatinous (transparent) DNA precipitates are obtained.	Dissolve gelatinous DNA into TE or H_2O. Add the High-Salt Precipitation Solution* at a half volume of this dissolved solution and stir well. Perform isopropanol precipitation by adding isopropanol at an equal volume of the High-Salt Precipitation Solution and stir well. Isopropanol to be added does not need to be cooled. Immediately after the addition, centrifuge at 10 K×g for 15 min to obtain DNA precipitates. * High-Salt Precipitation Solution: Code No. 313-06341 （1.2 M NaCl, 0.8 M Sodium citrate）
Yield is low.	・ Samples should be as fresh as possible. ・ mince the sample into 3 mm×3 mm or smaller pieces as finely as possible. ・ Scale up. ・ Mix by inverting a few times during the 50°C incubation period.
A large amount of RNA is contaminating the obtained DNA solution.	Add the attached RNase A to the obtained DNA solution to a final concentration of 10-20 μg/ml and react at 37°C for 30 min. If necessary, perform phenol/chloroform treatment after the reaction.
No amplification is found when PCR is performed using the obtained DNA as a template.	An improvement may be made by repeating the ethanol precipitation twice. In this case, use ethanol cooled at -20°C and centrifuge immediately.

VII Q & A

Q1. In the case of plant samples, the morphology of the sample does not change after adding Solution II.
A1. When processed in accordance with the normal protocol, if this is left immersed in Solution II for a long period of time, the morphology changes even though the change may not be determined by eye. However, when processed to this condition, the DNA solution finally obtained may contain a large amount of contaminants.

Q2. In the case of stiff leaves such as fruit trees, the DNA yield is low using the normal protocol.
A2. DNA may be extracted by freezing the sample with liquid nitrogen and pulverizing beforehand.

Q3. Can DNA be isolated from animal tissues/cells?
A3. No, generally speaking this kit is not suitable for that, although it has been confirmed that DNA can be isolated from shrimps. At the moment, DNA extraction using this kit is confirmed for plants, yeasts, bacteria, fungi and the like. (See p. 6, *VIII Data collection*.)

VIII Data collection

【 DNA extracted from plants 】

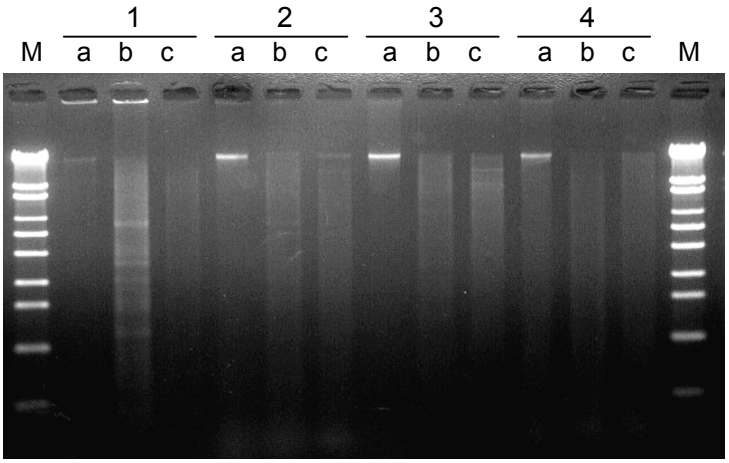
《 Yield and purity of extracted DNA 》

	ISOPLANT		CTAB* method	
	yield (μg/g)	$A_{260/280}$	yield (μg/g)	$A_{260/280}$
Arabidopsis thaliana	100-120	1.8	15-30	1.8
spinach	80-120	1.8	30-50	1.8
tobacco	4-20	1.8	60-80	1.7
tulip	10-20	1.8	10-20	1.8
rice	10-20	1.8	10-20	1.7

* CTAB: cetyltrimethylammonium bromide

《 Restriction enzyme reaction of extracted DNA 》

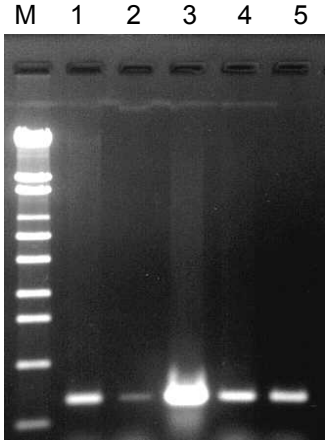
It has been confirmed that the extracted DNA can be cut with restriction enzymes (EcoR I and Hind III) using 2-20 times of the quantity.



lane
1 spinach
2 A*rabidopsis thaliana*
3 tobacco
4 rice
a: intact
b: EcoR I digest
c: Hind III digest
M: Marker 6 (λ/Sty I digest)

《 PCR of extracted DNA 》

It has been confirmed that the specific band can be amplified by PCR using the extracted DNA as a template and using the primers for amplifying the tobacco ribulose-1,5-diphosphate carboxylase large subunit gene.



lane
1 spinach
2 A*rabidopsis thaliana*
3 tobacco
4 tulip
5 rice
M: Marker 6 (λ/Sty I digest)

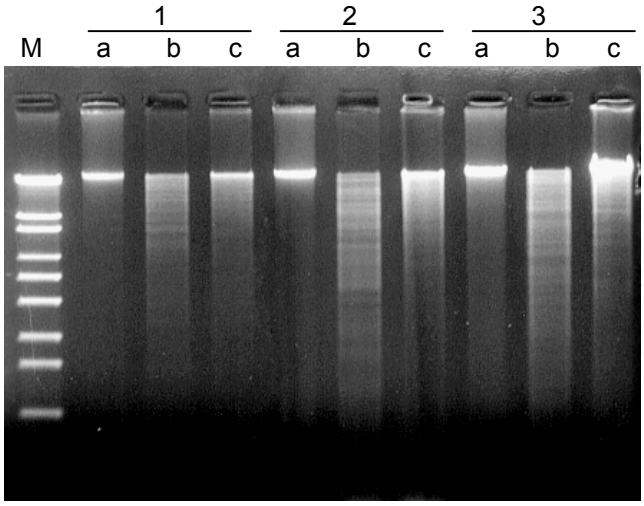
【 DNA extracted from bacteria 】

《 Yield and purity of extracted DNA 》

	ISOPLANT		phenol/chloroform method	
	yield (μg/g)	$A_{260/280}$	yield (μg/g)	$A_{260/280}$
Anabaena variabilis	125 – 500	1.8	60 – 500	1.7
Bacillus amyloiquefaciens	250 – 500	1.8	1000	1.8
Bacillus subtilis	100	1.8	—	—
Escherichia coli	125 – 500	1.8	250	1.8
Haemophilus influenzae	250 – 1000	1.8	250	1.7
Pseudomonas aeruginosa	80 – 125	1.8	—	—
Staphylococcus aureus	125	1.9	125	1.7
Thermus aquaticus	500	1.8	500	1.7
Xanthomonas holcicola	100	1.8	500	1.7

《 Restriction enzyme reaction of extracted DNA 》

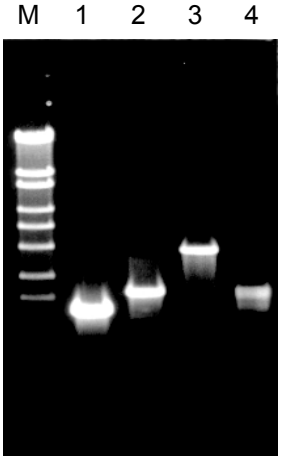
It has been confirmed that the extracted DNA can be cut with restriction enzymes (*Eco*R I and *Hin*d III) using 2-20 times of the quantity.



lane
1 *Escherichia coli* JM109
2 *Staphylococcus aureus* 3A
3 *Pseudomonas aeruginosa*
a: intact
b: EcoR I digest
c: Hind III digest
M: Marker 6 (λ/Sty I digest)

《 PCR of extracted DNA 》

It has been confirmed that the specific band can be amplified by PCR using the extracted DNA as a template.

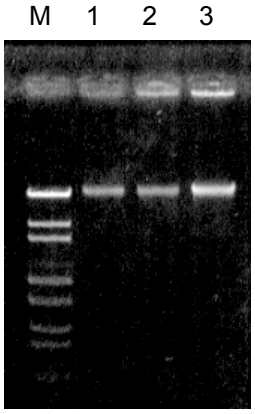


lane
1 *Escherichia coli* JM109
2 *Bacillus amyloiquefaciens*
3 *Bacillus subtilis*
4 *Staphylococcus aureus* 3A
M: Marker 6 (λ/Sty I digest)

【 DNA extracted from yeasts 】

《 Yield and purity of extracted DNA 》

	yield (μg/mg)	$A_{260/280}$
Saccharomyces cerevisiae	0.504	1.83
Shizosaccharomyces pombe	0.348	1.79
Phichia pastoris	2.523	1.87



lane
1 *Saccharomyces cerevisiae*
2 *Shizosaccharomyces pombe*
3 *Phichia pastoris*
M: Marker 6 (λ/Sty I digest)

【 DNA extracted from actinomycete and fungi 】

《 Yield and purity of extracted DNA 》

	yield (μg/g)	$A_{260/280}$
Mycrobacterium phlei	10.8	1.79
Nocardia asteroides	21.2	1.92
Streptomyces albus	3.3	2.22
Streptomyces sp.2-1	0.8	2.00
Streptomyces sp.MY-31	8.0	2.09
Neurospora sitophila	34.8	1.73
Rhizopus nigricans	23.0	2.04
Penicillium chrysogenum Q176	32.8	1.80
Aspergillus awamori	36.1	1.62
Aspergillus japonicus	20.5	1.90
Aspergillus niger	36.0	1.73
Aspergillus oryzae	6.8	2.04

IX References

1) Anil K.Jhingan,*Methods in Molecular and Cellular Biology*,**3**,**15**(1992)
2) Heng,Z.*et al*,*Nucleic Acids Research*,**21**(22),5279(1993)

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