Nippon Gene

Nucleic acid extraction from agarose gel

Thermostable β - Agarase

Code No. 317-07123（30 units）, 311-07121（300 units）

Manual (ver.4) 20120105KH

I Product description

Agarase is an enzyme which degrades melted (solated) agarose to neoagaro-oligosaccharides, while the agarose solution after the degradation does not form a gel again. This property allows Agarase to be used for extracting nucleic acid from agarose gel.

The advantages of nucleic acid extraction by agarase include the following: (1) manipulations are simple, (2) hazardous reagents are not used, (3) relatively large DNA fragments can be recovered without damage.

This product is an agarase derived from a heat-resistant microorganism and has excellent heat resistance compared to conventional agarase.

< Properties >

- Manipulations are simple and quick.
- The optimum reaction temperature is high, and this product can be used for standard agarase.
- The degraded gel solution can be used as-is for reactions such as cloning and restriction enzyme reactions.
- Large DNA fragments can be recovered with minimal shearing.

This enzyme is derived from a heat-resistant microorganism collected from the deep sea by the manned research submarine “Shinkai 6500” as part of research by the Extremobiosphere Research Program Institute of Biogeosciences Japan Agency for Marine-Earth Science and Technology. For more details, please refer to Chapter 5, Search for useful enzymes from deep sea microorganisms p.41-52 (authors: Yuji Hatada, Yukari Ohta, Yuko Hidaka, Yuichi Nogi) in “Current Technologies of Development and Application of Enzymes”, CMC Publications.

Provided by the Japan Agency for Marine-Earth Science and Technology

II Product content

Composition：Thermostable β-Agarase（30 units / 300 units）× 1

Activity：1 unit/ μl

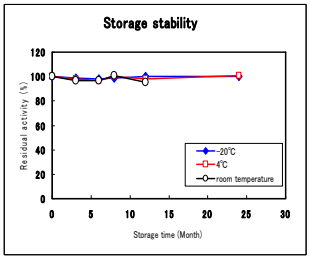
Storage Conditions：50 mmol/l NaCl, 20 mmol/l Tris-HCl (pH 7.5)

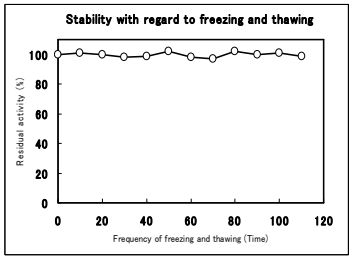
Definition of Units：

One unit is defined as an enzyme activity that produces reducing sugar equivalent amount of 1 μmol D-galactose from agarose gel per 1 min at 60°C.

III Storage

Storage temperature： refrigerated（2-10°C）





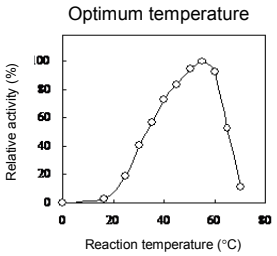
IV Protocol

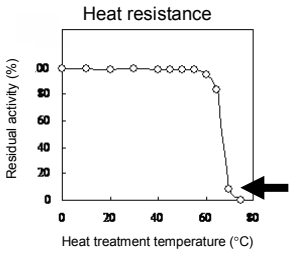
Basic protocol

(1) Put 200 mg (～200 μl) of excised agarose gel block in a tube.
↓
(2) Heat for 5-10 min to completely melt agarose gel. *1
↓
(3) After confirming that agarose is melted, transfer to a 50-65°C constant temperature bath (cool by standing for 30-60 sec). *2 *3
↓
(4) Add 6 units (6 μl）of Thermostable β-Agarase and mix. *4
↓
(5) Incubate at 50-65°C for 5-10 min to degrade agarose. *5
↓
(6) Allow to stand on ice to confirm that the solution does not re-coagulate. *6
(Perform alcohol precipitation/purification as necessary) *7

*1 The melting point of the gel depends on the kind of agarose and buffer.

*2 Thermostable β-Agarase demonstrates the maximum activity at 50-60°C (left figure). Also, Thermostable β-Agarase keeps 10% or higher activity after heating at 70°C for 30 min (right figure: arrow).





Data provided by the Japan Agency for Marine-Earth Science and Technology

*3 In the case of low melting point agarose (gel melting point of 65°C or lower, TAE being used) these steps (2)/(3) can be omitted.

*4 The amount of enzyme to be used can be reduced (see the section "VI Q&A").

*5 The reaction time varies depending on the amount of agarose gel block and the container size and the amount of Thermostable β-Agarase being added.

*6 If no coagulation is observed in ice cold water, it can be determined that agarose is completely degraded. When recovered DNA solution is to be used for electrophoresis, the enzyme should be inactivated by heat treatment (90°C, 5 min) or by a phenol/chloroform treatment.

*7 If undegraded gel remains, remove the gel by centrifuge, or restart the reaction from the start of the protocol. If necessary, perform alcohol precipitation/purification.

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V Troubleshooting

Problems and countermeasures

Undegraded gel remaining

Insufficient reaction of Thermostable β-Agarase may be the cause.

1. Add Thermostable β-Agarase after confirming that the gel is completely melted in step (2) (gel-dissolving reaction) of the basic protocol by pipetting, etc.
2. Increase the amount of Thermostable β-Agarase to be added.
3. Extend the reaction time of the gel degradation after adding Thermostable β-Agarase.

Low purity of recovered DNA

High molecular weight degradation products by agarose may remain.

1. Completely degrade the gel.
2. Precipitate the undegraded substance by centrifuge and use the supernatant as the DNA solution.
3. Perform alcohol precipitation/purification. Perform phenol/ chloroform treatment for further purification.

Thinning of the gel around the well in the electrophoresis of the recovered DNA

Thermostable β-Agarase may be persisting.

1. Inactivate by performing a heat treatment at 90°C or higher for 5 min or longer.
2. Perform phenol/chloroform treatment.

VI Q & A

Q1. Can the amount of the enzyme to be employed be reduced?

A1. The amount of the enzyme recommended by the standard protocol of Thermostable β-Agarase is 6 µl (6 units) per 200 mg gel block, but the amount of the enzyme to be employed for one reaction can be reduced depending on the gel concentration and the reaction condition.

Reaction time	Agarose concentration	Amount of enzyme added
5 min treatment (agarose gel：200 mg)	1% Agarose S	2 µl (2 units)
	1.5% Agarose S	3 µl (3 units)
	2% Agarose S	5 µl (5 units)
	1.5% Agarose XP	3 µl (3 units)
10 min treatment (agarose gel：200 mg)	1.5% Agarose S	1.5 µl (1.5 units)
	2% Agarose S	3 µl (3 units)
	3% Agarose 21	5.5 µl (5.5 units)

Q2. What is the rate of DNA recovery?

A2. In principle, all DNA in the excised gel is recovered.

Q3. What is the final volume of the reaction solution?

A3. When 200 mg of agarose gel is used, the final volume of the solution is about 200 µl.

Q4. How can the reaction solution be concentrated?

A4. Perform alcohol precipitation.

Example: Add 0.1 volume of 3 mol/l sodium acetate to the obtained DNA solution (degraded gel solution) (add the coprecipitating agent, Ethachinmate as necessary, and mix). Add 0.8-1 volume of isopropanol (or 2.5 volumes of ethanol), mix, leave standing at room temperature for 2-10 min, centrifuge (12 K X g, 10 min) and remove the supernatant. Add about 1 ml of 70% ethanol to the tube containing the precipitates, centrifuge (12 K X g, 5 min) and remove the supernatant. Leave the precipitates standing at room temperature for 15 min to dry and then dissolve into an appropriate amount of TE buffer.

Q5. Can high molecular weight DNA fragments be recovered?

A5. We have confirmed that large DNA fragments can be recovered with much less physical shearing (confirmed by recovering DNA fragments of tens of bp to 166 kbp) with Thermostable β-Agarase. However, the bigger the size of the DNA to be manipulated, the more it is difficult to separate by agarose gel electrophoresis, and the more prone it will be to shearing by manipulations such as pipetting.

Q6. Can the reaction solution be used for PCR reactions?

A6. We have confirmed that the solution can be used for PCR reactions by adding not more than 1/8 volume of the agarose solution degraded by Thermostable β-Agarase.

Example: When the volume of the PCR reaction solution is 20 µl, add 2.5 µl or less of the degraded agarose solution.

Q7. Can the reaction solution be used for cloning?

A7. We have confirmed that DNA solution recovered by Thermostable β-Agarase can be used as-is for ligation and transformation. (Confirmed by using the Ligation - Convenience Kit and *ECOS*™ Competent *E. coli* DH5α.)

Q8. Can the reaction solution be used for transcription reactions?

A8. We have confirmed that DNA solution recovered with Thermostable β-Agarase can be used as-is for transcription reaction. (Confirmed by using the *CUGA®7 in vitro* Transcription Kit)

Q9. Can the reaction solution be used for sequencing?

A9. We have confirmed that DNA solution recovered by Thermostable β-Agarase can be used as-is for sequencing.（Confirmed by using a 377 Sequencer, ABI Inc,）

However, if high-concentration/high molecular weight degraded products of agarose is mixed in, there may be problems depending on the model of the analytical instrument. High molecular weight degraded products of agarose gel can be removed with a phenol/chloroform treatment.

Q10. Can this product be used for extracting RNA from denaturing agarose gel?

A10. It can be used, but since formaldehyde included in denaturing agarose gel block inhibits the enzyme activity, the amount of the enzyme to be added needs to be increased.

Example: After melting 100 mg of 1.5% Agarose S denaturing gel block at 90°C for 5 min, agarose is degraded by adding 8 µl (8 units) of the enzyme and reacting at 60°C for 10 min.

For more details on experimental examples and the like, see the website of Nippon Gene.

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