NIPPON GENE

Recovery of nucleic acids from agarose gels

Thermostable β-Agarase

Catalog No.	Product	Size
317-07123	Thermostable β-Agarase	30 units
311-07121		300 units

For research use only.

β-agarase is an enzyme which hydrolyzes β-1,4 linkages in agarose to produce neoagaro-oligosaccharides. Agarose digested by β-agarase does not gelate again, therefore, nucleic acids can be recovered from agarose gels. Thermostable β-Agarase has a higher thermostability and stronger hydrolyzing activity than conventional β-agarase. Furthermore, a simple protocol allows for quick DNA and RNA purification. This enzyme is particularly suitable for purification of intact large DNA.

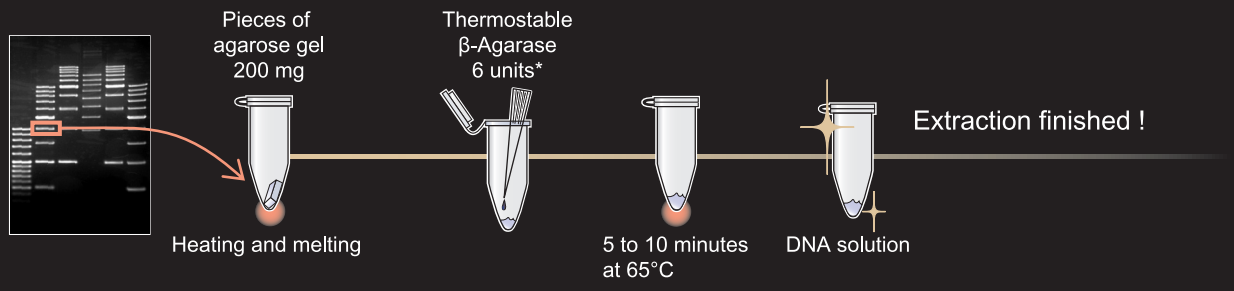
This enzyme was isolated from an agar-degrading bacterium living in a deep-sea by the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) using the submergible SHINKAI6500.

Activity: 1,000 units/ml
Unit difinition: 1 unit of Thermostable β-Agarase produces reducing sugar equivalent to 1 µmol of D-galactose in 1 minutes at 60°C
Form: 20 mM Tris-HCl (pH 7.5 at 25°C)
50 mM NaCl
Storage condition: Store at 2 to 8°C.

Overview

> Simple and short protocol (Reaction is completed in only 10 minutes).
> Can be used on standard agarose as well as low melting point agarose.
> Hydrolyzing gel solution is directly available for various applications. Such as cloning, restriction endonuclease digestion, sequencing, etc.
> Can effectively extract intact large DNA from agarose gels.

Procedure



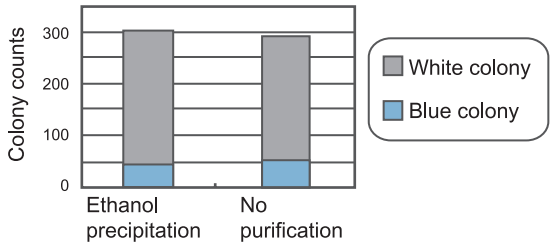
*The amount of an enzyme may be reduced by the density of the agarose gel.

Data

Cloning of the DNA recovered from agarose gel pieces.

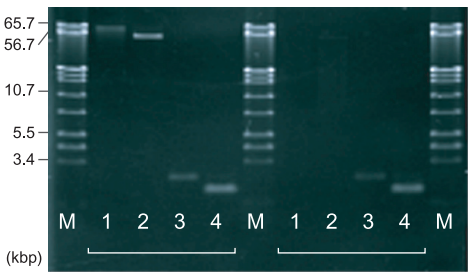
The 500 bp DNA fragment derived from λDNA was amplified by PCR.
PCR product were separated by 3% Agarose 21 (low molecular weight separation; NIPPONGENE).
A required band was excised form the gel with a razor.
After melting gel piece, it was treated by Thermostable β-Agarase.
9 µl of the gel solution was added to 1 µl of cloning vector DNA (3 kbp, 50 ng/µl) to set up ligation reaction using Ligation-Convenience Kit (NIPPON GENE).
After ligation reaction, *ECOS*™ Competent *E.coli* DH5α (NIPPON GENE) were transformed and then the number of colonies were counted. As the control, DNA solution purified by ethanol precipitation was used for ligation reaction.

Cloning efficiency



Extraction of intact large DNA from agarose gels.

T4 GT7 DNA (166 kbp), λDNA (48.5 kbp), pUC19 DNA (2.69 kbp) separated by agarose gel electrophoresis in 1x TAE was excised from 0.3% Agarose L (low-melting point; NIPPON GENE). The gel pieces were treated by Thermostable β-Agarase or spin column and the collected amount of DNA was compared.
One third amount of DNA was separated using 0.3% Agarose H (high gel strength; NIPPON GENE).



M: Marker 8 GT (0.4 µg)
1: T4 GT7 DNA
2: λDNA
3: pUC19 DNA (open circular)
4: pUC19 DNA (closed covalently circular)

Questions and Answers

Q1. How amount of Thermostable β-Agarase should be used ?
A1. Generally, add the enzyme at a ratio of 6 unit (6 µl) per 200 mg agarose gel. The amount of the enzyme can sometimes be reduced by the density of the gel and the condition.

- The amount of Thermostable β-Agarase which is needed for agarose gel to be hydrolyzed in 5 minutes

Agarose gel: 200mg

Agarose conc.	Enzyme volume
1.0% Agarose S	2.0 µl (2.0 unit)
1.0% Agarose S	3.0 µl (3.0 unit)
2.0% Agarose S	5.0 µl (5.0 unit)
1.5% Agarose XP	3.0 µl (3.0 unit)

- The amount of Thermostable β-Agarase which is needed for agarose gel to be hydrolyzed in 10 minutes

Agarose gel: 200 mg

Agarose conc.	Enzyme volume
1.5% Agarose S	1.5 µl (1.5 unit)
2.0% Agarose S	3.0 µl (3.0 unit)
3.0% Agarose 21	5.5 µl (5.5 unit)

Related agarose products

Agarose	Melting point	Chracteristic
Agarose S	≦90°C (1.5%)	Standard agarose
Agarose HS	≦93°C (1.5%)	High gel strength type of Agarose S
Agarose 21	≦85°C (3.0%)	Low molecular weight separation
Agarose XP	≦70°C (3.0%)	Low-melting point and low molecular weight separation
Agarose X	≦93°C (4.0%)	High gel strength and low molecular weight separation
Agarose H	Boil (1.5%)	High gel strength and low molecular weight separation
Agarose L	≦65°C (1.5%)	Low-melting point
Agarose GB	≦65°C (1.5%)	For pulsed field gel electrophoresis

Q2. How can it be confirmed whether gel is completely hydrolyzed ?
A2. When not gelating even if DNA solution is cooled on the ice, agarose gel is completely hydrolyzed.

Q3. What is an advantage of thermostability ?
A3. It is not only used for standard gel, but can be used for low-melting point gel. Reaction finishes in only 10 minutes.

Q4. How much is recovery efficiency of a DNA ?
A4. Almost all of DNA can be recovered in general.

Q5. Thermostable β-Agarase maintains the enzyme activity at a long term ?
A5. This enzyme is stable for 2 years at 4 or -20 ℃. When keeping in the room temperature, it's stable for 1 year. The activity is not altered during one-hundred cycles of freezing and thawing.

Q6. What amount of hydrolyzation solution produces ?
A6. When using 200 mg agarose gel, the amount of hydrolyzed solution will be 200 µl.

Q7. How should hydrolyzed solution be concentrated?
A7. DNA can be concentrated by ethanol precipitation.

Q8. Can hydrolyzed solution be used for *in vitro* transcription reaction ?
A8. It's possible to synthesize RNA by using hydrolyzed solution directly as the template. *CUGA*®*7 in vitro* Transcription Kit (NIPPON GENE) was used for RNA synthesis.

Q9. Can hydrolyzed solution be used for DNA sequencing ?
A9. It's possible to sequence by using hydrolyzed solution directly as the template.

Q10. Is it possible to recover RNA from denaturing agarose gel by using Thermostable β-Agarase?
A10. It can be used, however formaldehyde inhibits the enzyme activity. Therefore, it's necessary to increase the amount of the enzyme.

Information

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