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HiYield™ Beta-Agarase

Description

HiYield™ Beta-Agarase is an enzyme that will completely digest the agarose polysaccharide core into neoagaro-oligosaccharides. Agarose digested by HiYield™ Beta-Agarase does not gel again; therefore, nucleic acids can be gently yet efficiently recovered from agarose gels. HiYield™ Beta-Agarase is derived from a heat-resistant microorganism; therefore it has excellent heat resistance in comparison with conventional agarase. Unlike most agarase can only be used in low melting point agarose, HiYield™ Beta-Agarase can be used in both low melting point agarose and standard agarose. Manipulations are simple and quick. Whole process can be completed in 10 minutes. Recovery can be up to 99%. HiYield™ Beta-Agarase is ideal for isolation of both long (> 30 kb) and short (< 100 bp) DNA/RNA fragments in gels. Especially, relatively large DNA fragments can be recovered without damage. Furthermore, the degraded gel solution can be used as-is for reactions such as cloning and restriction enzyme reactions.

Features

- Large DNA fragments can be recovered without damage.
- Can be used in both low melting point agarose and standard agarose.
- Whole process can be completed in 10 minutes. Recovery can be up to 99%.
- Degraded gel solution can be used as-is for reactions such as cloning and restriction enzyme reactions.

Specifications

Cat. No.	Product Name	Specification
YBA030	HiYield™ Beta-Agarase, 30 units	HiYield™ Beta-Agarase (1U/ul): 30ul
YBA100	HiYield™ Beta-Agarase, 100 units	HiYield™ Beta-Agarase (1U/ul): 100ul

Storage Buffer

HiYield™ Beta-Agarase is supplied in 50 mmol/l NaCl, 20 mmol/l Tris-HCl (pH 7.5).

Definition of Activity Unit

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

Applications

Ideal for quantitative recovery of DNA or RNA from both low melting point agarose and standard agarose. Degraded gel solution can be used as-is for reactions such as cloning and restriction enzyme reactions. Any remaining agarose oligosaccharides will not gel or interfere with subsequent DNA manipulations such as restriction digestion, ligation, cloning, labeling, sequencing or transformation.

Quality Control

HiYield™ Beta-Agarase is functionally tested by recovery of DNA fragments from gels.

Shipping and Storage Conditions

HiYield™ Beta-Agarase shall be shipped and stored at 2-8℃. With proper storage, HiYield™ Beta-Agarase can be stored for up to 24 months without showing any deduction in performance and quality. The activity is not altered during 100 cycles of freezing and thawing.

Protocol

Important Notes Before Starting:

For typical 200mg (200ul) of 1% agarose gel, a ratio of 2 units of HiYield™ Beta-Agarase shall be added. The amount of HiYield™ Beta-Agarase can be reduced or increased depending on the gel concentration and the reaction condition.

Reaction Time	Agarose Concentration	Amount of HiYield™ Beta-Agarase Added
5 minute treatment (Agarose gel: 200 mg)	1% Agarose S	2 µl (2 units)
	1.5% Agarose S	3 µl (3 units)
	1.5% Low Melting Point Agarose	3 µl (3 units)
	2% Agarose S	5 µl (5 units)
10 minute 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)

Notes:

1. Agarose Melting Point Table.

Agarose Type	Melting Point	Characteristics
Agarose HS	≦93℃ (1.5%)	High gel strength type of Agarose S.
Agarose X	≦93℃ (4.0%)	High gel strength and low molecular weight separation.
Agarose S	≦90℃ (1.5%)	Standard agarose.
Agarose 21	≦85℃ (3.0%)	Low molecular weight separation.
Agarose XP	≦70℃ (3.0%)	Low melting point and low molecular weight separation.
Agarose L	≦65℃ (1.5%)	Low melting point.
Agarose GB	≦65℃ (1.5%)	For pulse field electrophoresis.

2. When 200 mg of agarose gel is used, the final volume of the solution is about 200 µl.
3. Hydrolyzed solution can be used directly as the template for in vitro transcription reaction.

Procedures For Typical 200mg (200ul) of 1% Agarose Gel

Note: Step 2 and 3 can be omitted if low melting point (≦65℃) agarose gel in TAE buffer is used.

The total reaction time can be finished in less than 10 minutes.

Step 1	**Transfer up to 200mg (200ul) of gel slice to a 1.5 ml microcentrifuge tube.**
Step 2	**Heat for 5-10 minutes to completely melt agarose gel.** The melting point of the gel varies according to the kind of agarose and buffer used.
Step 3	**While the agarose gel is completely melted, transfer the tube to a 50-65℃ heating Instrument (such as heat block or water bath) for 30~60 seconds.** HiYield™ Beta-Agarase demonstrates the maximum activity at 50-60℃. The activity of HiYield™ Beta-Agarase is maintained even if it's heated at 70℃ for 30 minutes.
Step 4	**Add 2 units (2 µl) of HiYield™ Beta-Agarase.**
Step 5	**Incubate at 50-65℃ for 5~-10 minutes to digest aga rose.** The reaction time varies depending on the size of gel slice, size of the tube and the amount of HiYield™ Beta-Agarase added.
Step 6	**Let stand on ice for 5 minutes to confirm that the solution does not re-coagulate.** If the solution does not gel on ice, it can be determined that agarose is completely hydrolyzed. If undegraded gel remains, remove the gel by centrifuge, or repeat the protocol. In step 5, extend the incubation time to avoid low recovery of DNA/RNA.

Troubleshooting

Problem	Possible Reasons/Solution
Incomplete digestion of agarose	**The gel melting step is incomplete.** Make sure that agarose gel is melted completely before adding HiYield™ Beta-Agarase. **The amount of HiYield™ Beta-Agarase added is insufficient.** Add more HiYield™ Beta-Agarase. **Incubation time is too short for Beta-Agarase to completely digest agarose.** Extend the incubation time in step 5.
Low purity of recovered DNA	**Residue of large quantities of neoagaro-oligosaccharides.** 1. Make sure that agarose gel is digested completely. 2. Remove the long neoagaro-oligosaccharides by centrifugation. 3. Purify DNA by ethanol precipitation. If necessary, perform phenol/chloroform treatment before ethanol precipitation.