β-Agarase I

NEW ENGLAND BioLabs Inc.
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M0392S 027121014101

M0392S

100 units **1,000 U/ml** **Lot: 0271210**

RECOMBINANT **Store at –20°C** **Exp: 10/14**

Description: β-Agarase I cleaves the agarose subunit, unsubstituted neoagarobiose [3,6-anhydro-α-L-galactopyranosyl-1-3-D-galactose] to neoagaro-oligosaccharides (1).

Source: Isolated from a strain of *E. coli* that carries a plasmid which encodes the β-Agarase I gene.

Supplied in: 50 mM Bis Tris-HCl (pH 6.5), 1 mM Na_2EDTA and 50% glycerol.

Reagents Supplied with Enzyme:
10X β-Agarase I Reaction Buffer.

Reaction Conditions: 1X β-Agarase I Reaction Buffer. **Incubate at 42°C**.

1X β-Agarase I Reaction Buffer:
10 mM Bis Tris-HCl
1 mM EDTA
pH 6.5 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to digest 200 µl of molten low melting point or NuSieve agarose to nonprecipitable neoagaro-oligosaccharides in 1 hour at 42°C.

Applications: β-Agarase I digests agarose, releasing trapped DNA and producing carbohydrate molecules which can no longer gel. The remaining carbohydrate molecules and β-Agarase I will not, in general, interfere with subsequent DNA manipulations such as restriction endonuclease digestion, ligation and transformation. Inhibition of DNA Polymerase I (Klenow Fragment) has been observed. β-Agarase I can be used to purify both large (> 50 kb) and small (< 50 kb) fragments of DNA from gels, and the resulting carbohydrates can be removed if necessary.

Heat Inactivation: Incubation at 95°C for 2 minutes or incubation at 65°C for 15 minutes inactivates 50 units of β-Agarase I. β-Agarase I retains activity for several hours at 45–50°C and is stabilized by the presence of agarose in the reaction.

Quality Control Assays

16-Hour Incubation: Incubation of 16 units for 16 hours at 42°C in 50 µl 1X β-Agarase I Buffer in the presence of 10 mM $MgCl_2$ and 1 µg phage λ DNA showed no DNA degradation.

Exonuclease Activity: Incubation of 10 units for 4 hours at 42°C in 50 µl 1X β-Agarase I Buffer supplemented with 10 mM $MgCl_2$ and 1 µg sonicated ^3H DNA (10^5 cpm/µg) released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 8 units with 1 µg ϕX174 RF I DNA for 4 hours at 42°C in 50 µl 1X β-Agarase I Buffer supplemented with 10 mM $MgCl_2$ gave < 10% conversion to RF II.

Ribonuclease Activity: Incubation of 4 units with 2 µg of NEB's RNA Molecular Weight Marker (NEB #N0362S) for 1 hour at 42°C in 50 µl 1X β-Agarase I Buffer followed by agarose gel electrophoresis gave no change in banding.

Agarose Digestion: Equilibrate the DNA-containing low melting point agarose (SeaPlaque GTG or NuSieve GTG) by washing the solid gel slice twice with 2 volumes of 1X β-Agarase I Buffer on ice for 30 minutes each.*

Remove the remaining buffer and melt the agarose by incubation at 65°C for 10 minutes. Cool to 42°C and incubate the molten agarose with 1 unit of β-Agarase I at 42°C for 1 hour. This procedure will digest up to 200 µl of 1% low melting point agarose. For larger volumes, adjust enzyme accordingly.

*As an alternative method of equilibration, add 1/10 volume of 10X β-Agarase I Buffer and melt together with the agarose. This faster equilibration method requires the amount of enzyme used to be doubled. This method is recommended

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when working with DNA fragments shorter than 500 base pairs because it avoids diffusion of DNA during washing.

Isolation of DNA

For Small DNA Fragments: DNA is precipitated while carbohydrates remain in solution.

1. Adjust the salt concentration of the β-Agarase I treated solution for isopropanol precipitation of DNA (0.5 M NaCl, 0.3 M NaOAc, 2.5 M NH_4OAc or 0.8 M LiCl).
2. Chill on ice for 15 minutes.
3. Centrifuge at 15,000 X g for 15 minutes to pellet any remaining undigested carbohydrates.
4. Remove the DNA-containing supernatant. Precipitate with 2 volumes of isopropanol. To ensure quantitative yields of small quantities of DNA (< 100 ng), carrier RNA (1 µg) can be added to the solution.
5. Mix thoroughly, chill and centrifuge at 15,000 X g for 15 minutes.
6. Remove the supernatant, wash the pellet with cold 70% isopropanol and dry the pellet at room temperature.
7. The pellet can be resuspended in TE or any buffer necessary for subsequent manipulation.

For Large DNA Fragments: Fragments larger than 50 kb require delicate handling to avoid mechanical shearing. Consequently, we advise that subsequent manipulation be carried out in the digested agarose or that a drop dialysis step be introduced to remove carbohydrates and β-Agarase I (MW 30,000).

Notes on Use:

1. Only low melting point agarose is suitable for β-Agarase I digestion as the solution must be liquid at the incubation temperature of 42°C. If the temperature falls below 42°C during the reaction time, even low melting point agarose will begin to congeal and be undigestable.
2. β-Agarase I is quickly inactivated at temperatures above 45°C. Therefore, when working with large volumes, be sure to leave ample time for the molten agar to equilibrate to 42°C.
3. β-Agarase I works best on gels made with Tris-acetate buffer (TAE). For gels made with Tris-borate buffer (TBE), doubling the required amount of β-Agarase I is recommended.
4. β-Agarase I works most efficiently on solutions containing 1% agarose or lower. For maximum digestion of higher percentage gels, melt the gel slice at 65°C and adjust the volume with 1X β-Agarase I Buffer so that the final concentration of agarose is 1%.
5. β-Agarase I exhibits optimal activity at pH 6.5. Greater than 75% of the optimal activity is maintained between pH 5.0–8.5.
6. Incubation at 95°C for 2 minutes or incubation at 65°C for 15 minutes inactivates 50 units of β-Agarase I. β-Agarase I retains activity for several hours at 40–45°C and is stabilized by the presence of agarose in the reaction.

References:

1. Yaphe, W. (1957) *Can. J. Microbiol.* 3, 987–993.
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