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ß-Agarase

EC 3.2.1.81 from *Pseudomonas atlantica*

Product	Cat#	Package size
ß-Agarase	**S5223.0050**	**50 units**
ß-Agarase	**S5223.0100**	**100 units**

Product description

ß-Agarase has been purified from an E. Coli strain carrying the cloned gene encoding ß-Agarase from Pseudomonas atlantica. The enzyme specifically digests the agarose polysaccharide core made up of repeating 1,3-linked ß-D-galactopyranose and 1,4-linked 3,6-anhydro-a-L-galactopyranose into neagaro-oligosaccharides. ß-Agarase from Pseudomonas atlantica is used for the isolation of DNA and RNA from “low-melt” agaroses. This technique allows the isolation of even large DNA fragments (> 10 kb), as there is almost no shearing compared to all/most other techniques.

ß-Agarase is active in a pH range from 5.0 to 8.5 with an optimum at 6.0. the optimal temperature is 40-42°C. Raising the temperature to 45°C accelerates the reaction, but reduces stability of the enzyme. Above 50°C ß-agarase is rapidly inactivated. The recovered nucleic acids can be subjected to many further manipulations such as restriction digest, ligation, labeling, sequencing, amplification, etc..

After digestion the DNA and RNA, respectively, is isolated by ethanol precipitation. To prevent a co-precipitation of agarose-oligosaccharides, perform the precipitation at room temperature with ammonium acetate (instead of sodium acetate). **NOTE:** Ammonium ions wil inhibit polynucleotide kinase at concentrations above 7 mM. In the case, that isolated DNA has to be phosphorylated, use sodium acetate.

The following agaroses from Genaxxon are suited for digestion by ß-agarase: M3046 GenAgarose Tiny; M3048 GenAgarose LMH; M3045 GenAgarose Plus.

Storage buffer

The enzyme is supplied in storage buffer: 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 % Triton X-100, 50% Glycerol.

Unit definition

One ß-agarase unit completely degrades 100µL (appr. 100mg) of molten 1% agarose in 30 minutes at 42°C.

Specifications

Acitivity:	0.2 – 1 U/µL
DNAses/RNAses/Proteases:	not detectable
Phosphatases:	not detectable
MW:	32.7 kDa
CAS:	[37288-57-6]

Storage

Store after delivery at -20°C

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Genaxxon BioScience

ß-Agarase

Manual

Agarose digestion

1. Determine the weight of a microcentrifuge tube first.
2. Cut out the desired band from the agarose gel as precise as possible. Determine the weight of the agarose slice after transfer to the 1.5mL microcentrifuge tube and melt the agarose at 70°C completely.
   Attention: Incubation at elevated temperatures may denature the DNA. Digest no more than 200-500mg agarose per tube. Cut gel slices larger than 200mg into smaller pieces. If the agarose is completetly melted, the hydrolysis of the agarose wil be incomplete, too.
3. Transfer the tube to a water bath adjusted to 40-42°C for approximately 5 minutes to equilibrate to the optimum reaction temperature prior to adding agarase.
4. Add one unit of ß-agarase per 100µL (appr. 100mg) of 1% molten agarose and incubate for 30 minutes at 40-42°C. When using higher percentage agarose, the amount of agarase should be proportionaly increased.

Purification of large DNA fragments

1. DNA fragments largen than 30 kb must be handled very carefully to prevent mechanical shearing. Therefore, centrifuge at 15000xg for 10 minutes to pellet undigested carbohydrates.
2. Remove oligosaccharides and agarase by dialysis or carry out subsequent manipulations with DNA in the diegested agarose solution.

Purification of small DNA fragments

1. DNA fragments smaller than 30 kb can be isolated from the digested agarose by ethanol precipitation.
   Add salt to the hydrolysed agarose: Either ammonium acetate (final concentration 2.5 M) or sodium acetate (final concentration 0.3 M).
   Attention: To prevent co-precipitation of agarose-oligosaccharides, perform the precipitation at room temperature with ammonium acetate rather than sodium acetate. **NOTE:** T4 polynucleotide kinase is inhibited by ammonium ions at concentrations above 7 mM. Use sodium acetate, if the 5'-ends of the isolated DNA will be labelled with T4 Kinase.
2. Chill on ice for 5 minutes, centrifuge at 15000xg for 10 minutes to pellet undigested carbohydrates.
3. Transfer the supernatant to a clean new tube and either add 1 volume of isopropanol or 2-3 volumes of ethanol. Mix gently and incubate for at least 30 minutes at 0°C to 22°C.
   Attention: If the DNA fragments are smaller than 500 bp or if the DNA concentration is lower than 0.05 µg/mL, incubate overnight at 0°C to 22°C.
4. Centrifgue at 15000xg for 15 minutes. Remove the supernatant and dry pellet.
   Resuspend in an appropriate buffer for subsequent manipulation.

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ß-agarase activity in different buffer systems

150%	50 mM Bis-Tris, pH 6.5, 1mM EDTA	100 mM Bis-Tris, pH 6.5, 10 M EDTA	10 mM Tris, pH 7.6, 5 mM EDTA, pH 8.0, 0.1 M NaCl
120%	90 mM Tris-Phosphate (TBE-buffer), 2mM EDTA	40 mM Tris-Acetate (TAE-buffer), 1 mM EDTA	
100%	45 mM Tris-Borate (TBE-buffer), 1 mM EDTA		