Bacteroides Heparinase II

1-800-632-7799
info@neb.com
www.neb.com

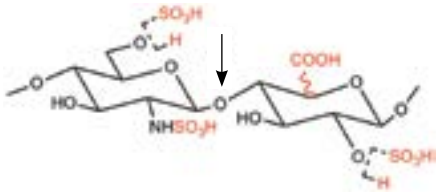
P0736S 003121013101

P0736S

80 units	**4,000 U/ml**	**Lot: 0031210**
RECOMBINANT	**Store at –80°C**	**Exp: 10/13**

Description: *Bacteroides* Heparinase II cloned from *Bacteroides eggerthii*, also called Heparin Lyase II, is a low specificity enzyme that is active on both heparin and heparan sulfate. The reaction yields oligosaccharide products containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm.

Detailed Specificity:



Denotes either glucuronic acid or iduronic acid.

All structural determinants for enzyme specificity are displayed in red.

Bacteroides Heparinase II specificity.

Similar to *Flavobacterium heparinum* Heparinase II, *Bacteroides* Heparinase II cleaves the glycosidic bond between *N*-sulfated and glucuronic or iduronic acid residues. When used alone this enzyme rarely yields complete depolymerization of a polysaccharide chain, however disaccharide analysis is enhanced when used in combination with Heparinase I and III.

Source: Cloned from *Bacteroides Eggerthii* and expressed in pACYC-T7-Ter.

Supplied in: 100 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C), 1 mM Na_2EDTA and 5 mM $CaCl_2$.

Reagents Supplied with Enzyme:
10X *Bacteroides* Heparinase Reaction Buffer

Reaction Conditions:

1. Combine 10 µl of 1 mg/ml heparin substrate, 10 µl *Bacteroides* Heparinase Reaction Buffer and H_2O in a total reaction volume of 100 µl.
2. Add 1 µl *Bacteroides* Heparinase II
3. Incubate reaction at 30°C for 1–24 hours (monitor absorbance at 232 nm for determination of partial or complete digestion).

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

1X *Bacteroides* Heparinase Reaction Buffer:
100 mM NaCl (pH 7.0 @ 25°C)
20 mM Tris-HCl
1.5 mM $CaCl_2$

Unit Definition: One unit is defined as the amount of enzyme that will liberate 1.0 µmol unsaturated oligosaccharides from porcine mucosal heparin per minute at 30°C and pH 7.0 in a total reaction volume of 100 µl.

Unit Definition Assay: Two fold dilutions of *Bacteroides* Heparinase II are incubated with 1 mg/ml porcine mucosal heparin substrate in 1X *Bacteroides* Heparinase Reaction Buffer, in a 100 µl reaction. The reaction mix is incubated at 30°C. Liberation of unsaturated oligosaccharides is detected by real-time UV spectroscopy at 232 nm.

Molecular Weight: 86,000 daltons.

Quality Assurance: No contaminating exoglycosidase, sulfatase, uronidase or proteolytic activity could be detected (ND).

Quality Controls

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection and mass spectrometry analysis.

(see other side)

CERTIFICATE OF ANALYSIS

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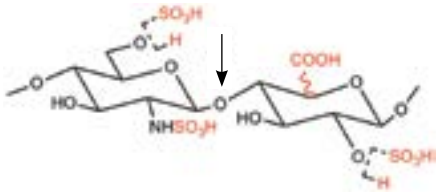

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CERTIFICATE OF ANALYSIS

Glycosidase and Sulfatase Assays:
8 units of *Bacteroides* Heparinase II were incubated with 0.1 mM of AMC (7-amino-4-methyl-coumarin) fluorescently-labeled oligosaccharides, in a 10 µl reaction for 20 hours at 30°C. The reaction products were analyzed by TLC for digestion of substrate.

No other glycosidase activities were detected (ND) with the following substrates:

β-*N*-Acetylglucosaminidase:
GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC ND

β-*N*-Acetylgalactosaminidase:
GalNAcβ1-4Galβ1-4Glc-AMC ND

β-Galactosidase:
Galβ1-3GlcNAcβ1-4Galβ1–4Glc-AMC ND
Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC ND

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-AMC ND

N,6-*O*-Sulfatase and Uronidase:
ΔUA-(1-4)-GlcNS6S-AMC ND

2-*O*-Sulfatase:
ΔUA2S-(1-4)-GlcNS6S-AMC ND

Protease Assay: After incubation of 40 units of *Bacteroides* Heparinase II with 0.2 nmol of a standard mixture of proteins for 20 hours at 37°C, no proteolytic activity could be detected by SDS-PAGE.

Note: Avoid repeated freeze-thaw cycles.

Heat Inactivation: 100°C for 1 minute.

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GalNAcβ1-4Galβ1-4Glc-AMC ND

β-Galactosidase:
Galβ1-3GlcNAcβ1-4Galβ1–4Glc-AMC ND
Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC ND

β-Glucosidase:
Glcβ1-4Glcβ1-4Glc-AMC ND

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