

Bacteroides Heparinase III



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



P0736S 003140215021

P0737S



14 units **700 U/ml** **Lot: 0021402**
RECOMBINANT **Store at -80°C** **Exp: 2/15**

Description: *Bacteroides* Heparinase III cloned from *Bacteroides eggerthii*, also called Heparin Lyase III, 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.

Bacteroides Heparinase III



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



P0736S 003140215021

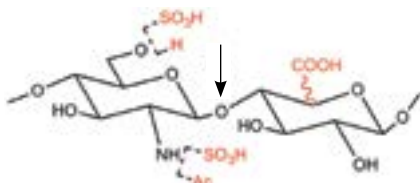
P0737S



14 units **700 U/ml** **Lot: 0021402**
RECOMBINANT **Store at -80°C** **Exp: 2/15**

Description: *Bacteroides* Heparinase III cloned from *Bacteroides eggerthii*, also called Heparin Lyase III, 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 III specificity.

In stark contrast to the *Flavobacterium heparinum* Heparinase III that cleaves the glycosidic bond only between hexosamines and glucuronic acid residues, the *Bacteroides* Heparinase III can cleave the glycosidic bond between hexosamines and either iduronic acid or glucuronic acid residues. *Flavobacterium heparinum* Heparinase III is not active on an *N*-sulfoglucosamine with 6-sulfation, whereas the *Bacteroides* Heparinase III is active in the presence of 6-sulfation.

Source: Cloned from *Bacteroides Eggerthii* and expressed in pET21A.

Supplied in: 100 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C), 1 mM Na₂EDTA and 5 mM CaCl₂.

Reagents Supplied with Enzyme:

10X *Bacteroides* Heparinase Reaction Buffer

Reaction Conditions:

1. Combine 10 µl of 1 mg/ml heparan sulfate, 10 µl *Bacteroides* Heparinase Reaction Buffer and H₂O in a total reaction volume of 100 µl.
2. Add 1 µl *Bacteroides* Heparinase III
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 CaCl₂

Unit Definition: One unit is defined as the amount of enzyme that will liberate 1.0 µmol unsaturated oligosaccharides from heparan sulfate 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 III are incubated with 1 mg/ml porcine mucosal heparan sulfate 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: 75,000 daltons.

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

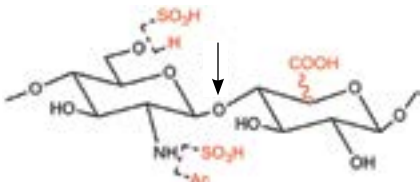
Quality Controls

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

Detailed Specificity:



Denotes either glucuronic acid or iduronic acid.

All structural determinants for enzyme specificity are displayed in red.

Bacteroides Heparinase III specificity.

In stark contrast to the *Flavobacterium heparinum* Heparinase III that cleaves the glycosidic bond only between hexosamines and glucuronic acid residues, the *Bacteroides* Heparinase III can cleave the glycosidic bond between hexosamines and either iduronic acid or glucuronic acid residues. *Flavobacterium heparinum* Heparinase III is not active on an *N*-sulfoglucosamine with 6-sulfation, whereas the *Bacteroides* Heparinase III is active in the presence of 6-sulfation.

Source: Cloned from *Bacteroides Eggerthii* and expressed in pET21A.

Supplied in: 100 mM NaCl, 20 mM Tris-HCl (pH 7.5 @ 25°C), 1 mM Na₂EDTA and 5 mM CaCl₂.

Reagents Supplied with Enzyme:

10X *Bacteroides* Heparinase Reaction Buffer

Reaction Conditions:

1. Combine 10 µl of 1 mg/ml heparan sulfate, 10 µl *Bacteroides* Heparinase Reaction Buffer and H₂O in a total reaction volume of 100 µl.
2. Add 1 µl *Bacteroides* Heparinase III
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 CaCl₂

Unit Definition: One unit is defined as the amount of enzyme that will liberate 1.0 µmol unsaturated oligosaccharides from heparan sulfate 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 III are incubated with 1 mg/ml porcine mucosal heparan sulfate 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: 75,000 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

Glycosidase and Sulfatase Assays: 1 unit of *Bacteroides* Heparinase III was incubated with 0.1 mM of AMC (7-amino-4-methyl-coumarin) fluorescently-labeled oligosaccharides, in a 10 μ l 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:
GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

β -N-Acetylgalactosaminidase:
GalNAc β 1-4Gal β 1-4Glc-AMC ND

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND
Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC ND

β -Glucosidase:
Glc β 1-4Glc β 1-4Glc-AMC ND

Page 2 (P0737)

Glycosidase and Sulfatase Assays: 1 unit of *Bacteroides* Heparinase III was incubated with 0.1 mM of AMC (7-amino-4-methyl-coumarin) fluorescently-labeled oligosaccharides, in a 10 μ l 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:
GlcNAc β 1-4GlcNAc β 1-4GlcNAc-AMC ND

β -N-Acetylgalactosaminidase:
GalNAc β 1-4Gal β 1-4Glc-AMC ND

β -Galactosidase:
Gal β 1-3GlcNAc β 1-4Gal β 1-4Glc-AMC ND
Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc-AMC ND

β -Glucosidase:
Glc β 1-4Glc β 1-4Glc-AMC ND

Page 2 (P0737)

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

2-O-Sulfatase:
 Δ UA2S-(1-4)-GlcNS6S-AMC ND

Protease Assay: After incubation of 5 units of *Bacteroides* Heparinase III 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.

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

2-O-Sulfatase:
 Δ UA2S-(1-4)-GlcNS6S-AMC ND

Protease Assay: After incubation of 5 units of *Bacteroides* Heparinase III 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.