Detailed Specificity:

Denotes either glucuronic acid or iduronic acid.

All structural determinants for enzyme specificity are displayed in red.

**Bacteroides Heparinase I specificity.**

In contrast to the *Flavobacterium heparinum* Heparinase I which cleaves the glycosidic bond between N-sulfated hexosamines and 2-O-sulfated iduronic acid residues, the *Bacteroides* Heparinase I cleaves between these same residues as well as the 2-O-sulfated glucuronic acid residues. The 2-O-sulfated uronic acid residue is essential for the activity of *Bacteroides* Heparinase I and 6-O-sulfation of GlcNS does not hinder enzyme activity. While *Bacteroides* Heparinase I cleaves 2-O-sulfated iduronic acid and 2-O-sulfated glucuronic acid residues at similar rates, the *Flavobacterium heparinum* Heparinase I has a much higher rate of cleavage for 2-O-sulfated iduronic acid residues (1). Limited digest of porcine mucosal heparin with *Flavobacterium heparinum* Heparinase I results in sulfated heparin oligosaccharides structures previously reported (2). Limited digest of porcine mucosal heparin with the *Bacteroides* Heparinase I results in heparin oligosaccharides with a lower extent of sulfation as reported (3).

**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 heparin substrate, 10 µl *Bacteroides* Heparinase Reaction Buffer and H₂O in a total reaction volume of 100 µl.
2. Add 1 µl *Bacteroides* Heparinase I.
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.

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**Bacteroides Heparinase I**

**240 units 12,000 U/ml Lot: 0021412 RECOMBINANT Store at –80°C Exp: 12/15**

**Description:** Bacteroides Heparinase I cloned from *Bacteroides eggerthii*, also called Heparin Lyase I, is active on heparin and the highly sulfated domains of heparan sulfate. The reaction yields oligosaccharide products containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm.
Quality Controls

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

Glycosidase and Sulfatase Assays:
24 units of *Bacteroides* Heparinase I 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:**
  - ∆UA-(1-4)-GlcNS6S-AMC ND

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

Protease Assay: After incubation of 120 units of *Bacteroides* Heparinase I 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.

References: