Detailed Specificity:

Denotes either glucuronic acid or iduronic acid.

**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 uronic acid residues at similar rates, the *Flavobacterium heparinum* Heparinase I has a much higher rate of cleavage for 2-O sulfated uronic acid residues (1). Limited digest of porcine mucosal heparin with *Flavobacterium* 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.

**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 I 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:** 42,000 daltons.

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

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**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.

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:

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>Substrate</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-N-Acetylglucosaminidase:</td>
<td>GlcNAcβ1-4GlcNAcβ1-4Glc-AMC</td>
<td>ND</td>
</tr>
<tr>
<td>β-N-Acetylgalactosaminidase:</td>
<td>GalNAcβ1-4Galβ1-4Glc-AMC</td>
<td>ND</td>
</tr>
<tr>
<td>β-Galactosidase:</td>
<td>Galβ1-3GlcNAcβ1-4Galβ1-4Glc-AMC</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC</td>
<td>ND</td>
</tr>
</tbody>
</table>

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: