UV spectroscopy at 232 nm.

**Bacteroides Heparinase I**

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 2-0 sulfated glucuronic acid residues at similar rates, the *Flavobacterium heparinum* Heparinase I has a much higher rate of cleavage for 2-O sulfated uronic acid residues.

**Detailed Specificity:**

Denotes either glucuronic acid or iduronic acid. All structural determinants for enzyme specificity are displayed in red.

**Bacteroides Heparinase I specificity.**

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**Detailed Specificity:**

Denotes either glucuronic acid or iduronic acid. All structural determinants for enzyme specificity are displayed in red.

**Bacteroides Heparinase I specificity.**

1. 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.

**Reaction Conditions:**

1. Combine 10 µl of 1 mg/ml heparin substrate, 10 µl *Bacteroides* Heparinase Reaction Buffer and H$_2$O 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).

**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.

**Quality Assurance:**

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

**Molecular Weight:** 42,000 daltons.

1. **Bacteroides Heparinase Reaction Buffer:**

   - 100 mM NaCl (pH 7.0 @ 25°C)
   - 20 mM Tris-HCl
   - 1.5 mM CaCl$_2$

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.

**Quality Assurance:**

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

**Molecular Weight:** 42,000 daltons.
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:**
  - ΔUA-(1-4)-GlcNS6S-AMC ND

- **2-O-Sulfatase:**
  - ΔUA2S-(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: