**Bacteroides Heparinase II**

**Recommended Use:**
- **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.

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

---

**Bacteroides Heparinase II**

**Recommended Use:**
- **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.

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

---

**Bacteroides Heparinase II**

**Recommended Use:**
- **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.

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

---

**Bacteroides Heparinase II**

**Recommended Use:**
- **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.

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

---

**Bacteroides Heparinase II**

**Recommended Use:**
- **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.

**Reagents Supplied with Enzyme:**
- 10X *Bacteroides* Heparinase Reaction Buffer
- 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 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.
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:**
  - Gicβ1-4Glcβ1-4Glc-AMC ND

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

- **2-O-Sulfatase:**
  - ∆UA2S-(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.