

DESCRIPTION

Source *E. coli*-derived
Gln22-Arg384, with an N-terminal Met and 6-His tag
Accession # Q05819

N-terminal Sequence Analysis Met

Predicted Molecular Mass 42 kDa

SPECIFICATIONS

SDS-PAGE 40-42 kDa, reducing conditions

Activity Measured by its ability to liberate oligosaccharides from heparin.
The specific activity is >24,500 pmol/min/μg, as measured under the described conditions.

Endotoxin Level <1.0 EU per 1 μg of the protein by the LAL method.

Purity >95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 μg per lane.

Formulation Supplied as a 0.2 μm filtered solution in Tris, NaCl and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Assay Buffer: 50 mM Tris, 100 mM NaCl, 2 mM CaCl₂, pH 7.5
 - Recombinant *F. heparinum* Heparinase I (rFhHeparinase I) (Catalog # 7897-GH)
 - Substrate: Heparin (Tocris, Catalog # 2812), 20 mg/mL stock in deionized water
 - 96-well Clear UV Plate (Costar, Catalog # 3635)
 - Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

- Assay**
1. Dilute rFhHeparinase I to 0.5 μg/mL in Assay Buffer.
 2. Dilute Substrate to 0.75 mg/mL in Assay Buffer.
 3. Load 100 μL of diluted rFhHeparinase I into a UV plate, and start the reaction by adding 200 μL of 0.75 mg/mL Substrate. Include a Substrate Blank containing 100 μL of Assay Buffer and 200 μL of 0.75 mg/mL Substrate.
 4. Read plate in kinetic mode for 5 minutes at an absorbance of 232 nm.
 5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* (\text{OD/min}) \times \text{well volume (L)} \times 10^{12} \text{ pmol/mol}}{\text{ext. coeff}^{**} (\text{M}^{-1}\text{cm}^{-1}) \times \text{path corr.}^{***} (\text{cm}) \times \text{amount of enzyme } (\mu\text{g})}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 3800 M⁻¹cm⁻¹

***Using the path correction 0.92 cm

Note: the output of many spectrophotometers is in mOD

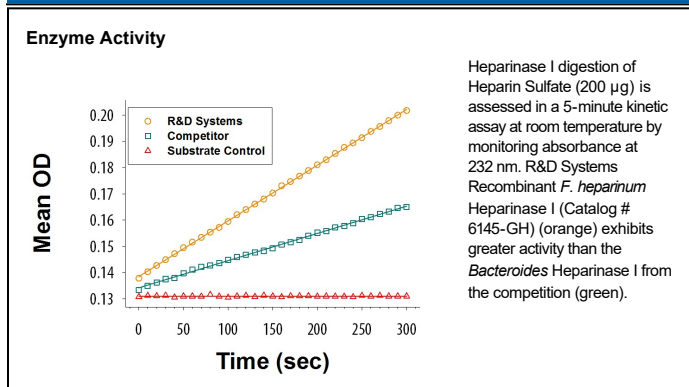
- Final Assay Conditions**
- Per Reaction:
- rFhHeparinase I: 0.05 μg
 - Substrate: 0.5 mg/mL

PREPARATION AND STORAGE

Shipping The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

- Stability & Storage** Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
- 6 months from date of receipt, -70 °C as supplied.
 - 3 months, -70 °C under sterile conditions after opening.

DATA



BACKGROUND

Heparin and heparan sulfate are sulfated glycosaminoglycans that share basic carbohydrate backbone structure with alternating uronic acid and N-acetylglucosamine residues (1, 2). Heparin is found in mast cells and has strong anticoagulation properties. Heparan sulfate is found on cell membrane and extracellular matrix and is involved in various biological events from cell growth, adhesion and migration to lipid metabolism. Heparin has a much higher degree of sulfation than heparan sulfate, which can be considered as a polysaccharide with regions similar to heparin interspaced with much less sulfated regions. Both heparin and heparan sulfate can be digested by heparinases, a group of bacterial lyases that are widely used as tools for processing and analyze these polysaccharides. Heparinases degrade heparin and heparan sulfate glycosaminoglycans through an eliminative mechanism (3). Heparinase I from Flavobacterium *heparinum* is highly active on heparin and has no activity against chondroitin sulfate and keratan sulfate (4). The enzyme readily releases highly sulfated oligosaccharides from heparin (5).

References:

1. MacArthur, J. M. *et al.* (2007) J. Clin. Invest. **117**:153.
2. Esko, J. D. and Selleck, S. B. (2002) Annu. Rev. Biochem. **71**:435.
3. Linhardt, R. J. *et al.* (1986) Appl. Biochem. Biotech. **12**:135.
4. Wu, Z.L. *et al.* (2011) Glycobiology **21**:625.
5. Ernst, S. *et al.* (1996) Biochem. J. **315**:589.